Discrimination of Biological Systems with Photon Emission: Steps Toward Diagnostics in Humans and Cell Cultures

by

Billy C. S. Yearington

Thesis submitted as a partial
requirement in the
Master of Science (M. Sc.) in Biology

Faculty of Graduate Studies

Laurentian University

Sudbury, Ontario

THESIS DEFENCE COMMITTEE/COMITÉ DE SOUTENANCE DE THÈSE
Laurentian Université/Université Laurentienne
Faculty of Graduate Studies/Faculté des études supérieures

Title of Thesis
Titre de la thèse — Discrimination of Biological Systems with Photon Emission: Steps Toward Diagnostics in Humans and Cell Cultures

Name of Candidate
Nom du candidat — Yearington, Billy

Degree
Diplôme — Master of Science

Department/Program
Département/Programme — Biology

Date of Defence
Date de la soutenance June 28, 2019

APPROVED/APPROUVÉ

Thesis Examiners/Examinateurs de thèse:

Dr. Blake Dotta
(Supervisor/Directeur de thèse)

Dr. Rob Lafrenie
(Co-supervisor/Co-directeur de thèse)

Dr. Mazen Saleh
(Committee member/Membre du comité)

Dr. Ankush Prasad
(External Examiner/Examinateur externe)

Approved for the Faculty of Graduate Studies
Approuvé pour la Faculté des études supérieures
Dr. David Lesbarrères
Monsieur David Lesbarrères
Dean, Faculty of Graduate Studies
Doyen, Faculté des études supérieures

ACCESSIBILITY CLAUSE AND PERMISSION TO USE

I, **Billy Yearington**, hereby grant to Laurentian University and/or its agents the non-exclusive license to archive and make accessible my thesis, dissertation, or project report in whole or in part in all forms of media, now or for the duration of my copyright ownership. I retain all other ownership rights to the copyright of the thesis, dissertation or project report. I also reserve the right to use in future works (such as articles or books) all or part of this thesis, dissertation, or project report. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that this copy is being made available in this form by the authority of the copyright owner solely for the purpose of private study and research and may not be copied or reproduced except as permitted by the copyright laws without written authority from the copyright owner.

ABSTRACT

Spontaneous biophoton emission has been observed in virtually all living organisms. Differences between individuals of the same species have not been documented thoroughly. The biophoton emission of both humans and cell cultures were measured. Two humans had their biophoton emission repeatedly measured over the course of five months. Significant spectral power density peaks were able to differentiate between the two human participants, but only in the first month of measurement. Multiple cell cultures, some non-malignant and some cancerous, had their biophoton emission measured in different sizes of containers filled with nutrient agar. Significant spectral power density peaks were observed between the B16-BL6 and C2C12 cell lines. B16-BL6 cells were exposed to an extremely low-frequency electromagnetic field for one hour. Their biophoton emission was measured immediately following exposure. Significant variability was observed in the spectral power density within specific frequency ranges. These results indicate that there exists temporal characteristics of biophoton emission that can be used to successfully discriminate within and between individuals. The potential for biophoton emission to be useful as a diagnostic tool is discussed.

ACKNOWLEDGEMENTS

I wish to thank Dr. Blake T. Dotta for all of his mentorship and guidance in my time with the Neuroscience Research Group. Without his confidence in me, I would not have succeeded in the completion of this document. I will always treasure our late afternoon conversations in Neurochemistry.

I would be remiss not to thank Dr. Michael A. Persinger. As someone who was fortunate enough to learn and study with his guidance, I will always be grateful for those few short few years. Thank you, Dr. P.

A big thank you to Dr. Robert M. Lafrenie and Dr. Carly A. Buckner for their assistance with various aspects of my journey.

To the members of the NRG, I was able to finish this journey thanks to your continued friendship and support. It is a group I will fondly cherish. Victoria, Trevor, Lucas, Ryan; your help in the lab will always be remembered.

A big thank you to all of my friends and family, for reminding me what I have back at home.

Finally, thank you to Ms. Shauna Stewart for her unending support through this chapter in my life. You are my world.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER TWO:

CHAPTER THREE:

CHAPTER FOUR:

LIST OF TABLES

CHAPTER TWO:

CHAPTER THREE:

CHAPTER ONE

INTRODUCTION

Photons are discrete quantities of light that are responsible for the transmission of the electromagnetic spectrum. It has been demonstrated that biological systems release photons as a result of their oxidative metabolism; this release is termed biophoton emission (Popp, 1994). This energy is typically within the order of $10^6 – 10^7$ photons/m^2/sec (Popp, 1979).

In this thesis, the observation of biophoton emission from human and cell cultures was investigated. Comparisons were made between human individuals as well as between a series of cell lines. These observations were conducted at the level of the organism, rather than focus on a specific area or individual cell. The impact of a novel electromagnetic field (EMF) was also assessed. All data was collected with the use of photomultiplier tubes (PMT). The PMTs were placed approximately 15cm away from the biological source for all experiments. All cell cultures were normalized to a size of approximately 10^6 cells before measurement.

The Dual Nature of Light

The definitions of light, and the material that composes it, have changed multiple times over the course of history, from speculation to theory-based calculations to experimental data to theory yet again. Perhaps one of the earliest recorded theories was that of the Vaisheshika school of Hindu philosophy, from ~500BCE. They proposed that light was arranged into rays of tejas, or fire atoms, and that the various properties of the light rays were derived from the characteristics of the tejas; for example, their speed and arrangement.

Two hundred years later, the Greek mathematician Euclid proposed the law of reflection. This law states that when light is reflected off of a surface the incident angle is the same as the reflected angle. This law came with two assumptions on the characteristics of light: one, that light travels in a straight line; and two, that these rays of light are discrete.

These ideas were expanded upon and consolidated by Arabian astronomer Ibn al-Haytham, considered the father of modern optics, in his 1021 book *Kitab al-Manazir* (*Book of Optics*). In this work, al-Haytham experimented with the laws of refraction with combinations of mirrors and lens. The aim of his work was to approximate the human eye. He proposed that the eye operates in a similar fashion to a pinhole camera, that light enters the eye rather than being emitted from it (a commonly accepted Greek theory), and that the image formed in the eye would be inverted. He deduced that the perception of vision occurs in the brain instead of the eye, and that vision is subjective. His work would be revived in the European Renaissance thanks to translations by English philosopher Robert Bacon.

In 1637, French philosopher René Descartes published *La dioptrique,* in which he proposed that light moved around spheres of aether (as he assumed there was no vacuum in nature) via instantaneous propagations. Similar theories were proposed during the rest of the 17th century, culminating with Dutch physicist Christiaan Huygens' work *Traité de la lumière* in 1690. He proposed that light was analogous to sound; it travelled though a medium via waves of continuous frequencies. Crucially, his work also explained the reflection and refraction of waves. Descartes' calculations were based on Huygens' principle, which states that each point of a wave front acts as a source for additional wavelets.

Here we see the emergence of the two competing theories of the nature of light: particle and wave. In 1704, English physicist Isaac Newton completed his work, *Opticks*; here, he suggested that light behaved as a particle. He proposed that colour should be related to either the mass or velocity of light particles, which would explain why different colours of light were refracted by different amounts.

The “final” triumph of particle theory came from the work of French physicist Augustin Fresnel, who, in the early 19th century, published both a mathematical wave theory of diffraction and how a wave of light could be polarized if it was a transverse wave with no longitudinal vibrations. The problem that still eluded physicists was the identification of the medium by which light travelled through, the “luminous aether”.

English physicist James Clerk Maxwell’s work was essential for laying the groundwork for our modern understanding of the properties of light. His work can be simplified into four partial differential equations: Gauss’ Law for electricity, Gauss’ Law for magnetism, Faraday’s Law, and Ampère’s Law.

Faraday’s Law and Ampere’s Law are incredibly important; together, they state that a changing magnetic field will produce a changing electric field, and that a changing electric field will produce a changing magnetic field. These two mechanisms can continue indefinitely, in a phenomenon known as propagation. From these equations, Maxwell predicted that the electromagnetic wave can travel without a medium due to propagation. He also accurately predicted the speed of this electromagnetic wave as 310 740 000m/s, which is only 0.0365% inaccurate from the currently accepted value of 299 792 458m/s.

At the dawn of the 20th century, physicists were still hunting for the “luminous aether” that was proposed to conduct waves of electromagnetic energy. In 1887, two American physicists, Albert Michelson and Edward Morley, had performed an experiment to detect this aether. They assumed that if light propagated through an aether perpendicular to Earth, its velocity would change due to interference with Earth’s velocity (Michelson & Morley, 1887). To measure this interference, a device later dubbed an interferometer was constructed. This device, shaped like an offset cross, sent a ray of light towards a set of mirrors. Here, the light was split and reflected at a

90° angle into the two arms. Each ray would then travel to the end of its arm, reflect off of a mirror located at that end, and travel back to the middle to be recombined and sent to an eyepiece.

If light interacted with an aether, as the light moved parallel to Earth, the "drag" produced by the movement of aether relative to Earth's movement against the velocity of light would produce a detectable interference pattern. This phase shift was estimated to be approximately 0.4 wavelengths; however, the highest detected phase shift was no less than 0.018 wavelengths in the first attempt, and after marked improvements in the efficacy of the device, the shift was no less than 0.02 wavelengths (Michelson & Morley, 1887). These results were a physical argument against the existence of a "luminous aether".

But, a problem remains. Maxwell's equations do not require that the velocity of the (charged) particle in question or the velocity of the observer be known in order to solve for the speed of the electromagnetic wave; it is a constant, c, which corresponds to the speed of light. Alternatively, Newtonian mechanics are based upon Galilean transformations; and these equations require the speed of the particle. One of these methods must be incorrect.

To illustrate this incompatibility, suppose that the particle is a pitcher and the wave is a baseball. If the pitcher is running forward at a speed v, and they throw the ball in the direction they are travelling, with Newtonian mechanics, the baseball travels at $c + v$. Maxwell would tell us that the speed of the ball would always be c, regardless of the speed of the pitcher.

At the end of the 19th century, there were still two theoretical problems that needed solving: what medium did light travel through? Does light follow Newtonian or Maxwellian equations? Work performed by the Dutch physicist Hendrik Lorentz would help solve these problems. Lorentz believed that light travelled though an aether. After hearing of the failure of the Michelson-Morley

experiments, he proposed an alternate view; the aether was fixed, and composed of charges that were excited by light. These charges would absorb and re-emit the light wave; mathematically, he was able to explain the Doppler effect, light aberration, and the results of the Fizeau experiment. The Fizeau experiment was similar to the Michelson-Morley experiment, but used water to show the interference of light rather than Earth's velocity.

To move between the stationary aether and moving systems, Lorentz provided a series of geometrical transformations, using a term which he called "local time". These equations would later be termed the Lorentz transformations, which finally united Newtonian and Maxwellian equations. Notably, at the time of their development, they were *ad-hoc*.

For some physicists, this was a satisfactory answer to the issue of light. For one German-Jewish physicist, Albert Einstein, the number of *ad-hoc* "hoops" that was necessary for the justification was too many. Einstein crucially rejected the "luminous aether", instead basing his work on the equations of Maxwell. His theory of special relativity, as it would later be known, was dependent on two postulates:

1. The laws of electrodynamics and optics are the same in all inertial frames of reference; and

2. The speed of light in a vacuum is constant for all observers.

Einstein's (1905) paper, *Zur Elektrodynamik bewegter Körper* (*On the Electrodynamics of Moving Bodies*), using the above postulates, was able to mathematically describe and derive all of the experimental results found by previous physicists. In addition, he was able to derive the Lorentz transformations, meaning they were no longer an *ad-hoc* explanation of the unity of Newton and Maxwell. This work did away with a "luminous aether"; it was not needed to explain the Lorentz contractions or the propagation of light.

Five years before Einstein's theory of special relativity, another German physicist, Max Planck, would also contribute to electromagnetic theory. In 1900, Planck was able to mathematically describe black body radiation, with a key distinction; the energy released was contained in discrete packages, or *quanta* (Planck, 1901). Einstein would later expand on this concept of *quanta* not only for black body radiation, but for all of the electromagnetic spectrum; he would term this concept *Lichtquant*. A later term, *photon*, would become the preferred name in English.

The first experimenter to demonstrate Einstein's and Planck's quantification of light was American physicist Arthur Compton. His 1923 experiment using x-rays produced a scattering effect that did not match with classical electromagnetic theory (Thomson scattering). Compton was able to derive the relationship between the wavelength shift and the scattering angle that was observed in his experiment. This relationship would later be termed Compton scattering.

A key assumption Compton made for this derivation is that one X-ray photon interacted with one electron (Compton, 1923). This disregarded classical electromagnetic theory that assumed the light wave would interact with multiple electrons and produce a Doppler-like shift in the observed frequency. Compton scattering was one of the first concepts to be explained with quantum electrodynamics and is incredibly relevant in today's medical field.

The Photon

Photons are discrete packages of energy, or *quanta*, that possess characteristics of both particles and wave functions. Photons have a range of frequencies in which they operate, typically in the range of 10^2 to 10^{22} Hz. The amount of energy per photon is dependent on its frequency; a relationship that is mediated by Planck's constant and the speed of light.

Photons are traditionally thought to travel at the speed of light. This supposes that a photon has a rest mass of zero. Recent experiments have suggested that the rest mass of the photon is a non-zero value, with an upper limit of approximately 10^{-54} kg (Goldhaber & Nieto, 2010). The implication of a photon with a non-zero rest mass may allow for alternative energies based on its velocity and mass rather than simply based on its wavelength.

Biophoton Emission

Photon emission, as a method of biological communication, was first proposed by Russian scientist Alexander Gurswitsch in 1922 (Bischof, 2005). His experimental set-up consisted of onion roots, separated by either normal glass or quartz glass. He found that the division of cells in one root tip would stimulate division in the opposing root tip, but only when separated by quartz glass, not normal glass (Bischof, 2005). He concluded that there must be a form of "mitogenentic radiation" that could pass through the quartz in the UV range; however, Gurswitsch was not able to measure this proposed method of information transfer reliably (Bischof, 2005). The introduction of photomultiplier tubes (PMTs) provided a reliable way to measure photon emission from biological systems. This process is also known as ultraweak photon emission (UPE), due to the low intensity of the light, which cannot be observed with common detectors, and is not visible to the naked eye (Van Wijk, 2005).

All living tissue produces some form of biophoton emission. This is distinct from bioluminescence in that no specialized organs or metabolic pathways are required for emission (Cifra & Pospisil, 2014). Biophoton emission is also not a consequence of thermal radiation. Inanimate objects heated to a temperature range of 30 °C to 90 °C produce no increases in UPE (Van Wijk, 2005). At 37°C, Plank's black-body radiation does not produce sufficient photons to match levels of measured biophoton emission (Cifra & Pospisil, 2014). The frequency of photons

emitted by biological systems are within the range of 250 – 800nm (Popp 2009). The specific intensities and frequencies vary for different cell types.

Two types of biophoton emission have been observed: spontaneous and induced. Spontaneous biophoton emission refers to the photons released by the organism at a normal or resting state, with no external influences or stimuli. Induced biophoton emission, by contrast, refers to the photons released by an organism after exposure to certain biotic or abiotic factors. These can include stress, mechanical damage, or ionizing radiation (Cifra & Pospisil, 2014). Induced biophoton emission is typically of greater intensity than spontaneous biophoton emission.

Metabolism is thought to be the common source of biophoton emission. The biophoton emission produced by metabolically inactive cells is very low or close to background levels. Metabolic pathways with oxidative reactions are proposed to be the main pathways associated with biophoton emission (Dotta *et al*., 2011). It was originally thought that biophoton emission was simply a consequence of metabolic activity with no meaningful effect on biological processes; however, recent experiments have shown that cells have the potential to "store" photons and release them after a certain period of time (Karbowski *et al*., 2016). This has led some to postulate that there is a biological relevance for biophoton emission, with roles in the regulation of growth and proliferation (Creath & Schwartz, 2005; Popp, 2009). Regardless of the biological relevance, biophoton emission remains a potential diagnostic tool. Takeda and colleagues were able to assess cancer cell populations with biophoton emission (1998).

Biophoton emission has also been observed in humans. Early studies were able to distinguish between the presence of a human from the background (Van Wijk, 2005). Human subjects were able to increase the amount of biophoton emission by using specific breathing techniques (Bischof, 2005). Edwards and colleagues systematically observed the UPE emitted

from the hand every 1.5h, for a total of 28h; they also observed UPE emitted from other regions, including, the forehead, abdomen, lower back, and chest (1989). They found the level of UPE was increased from the hand and forehead regions compared to the chest, abdomen, and lower back (Edwards *et al*., 1989). Popp and his colleagues were able to obtain information concerning the frequencies present within the UPE, significant light-right asymmetries, and the presence of a 24h cycle (Bischof, 2005). Longer cycles, such as a 1-4 week cycle, were also obtained from repeated measures of the hands and forehead (Cohen & Popp, 1997). Jung and colleagues have found seasonal variation in the biophoton emission from the hands (2004).

The estimated biophoton emission density for humans has been approximated to be 100 photons per cm^2 (Cohen & Popp, 1997). This intensity includes only photons with wavelengths from the visible spectrum; the potential for higher values due to infrared and ultraviolet photons is possible.

As with cells, biophoton emission from humans was also originally thought to be a by-product of metabolism with no biological relevance. Popp (1997) and others (Creath & Schwartz, 2005) have proposed that biophotons may act as signals for biological functions. Rahnama and colleagues have argued that biophotons may play a key role in neural signal propagation (2011). If biophoton emission has biological consequences, this may be useful in diagnostic approaches. The activity of various cellular-signalling pathways could be monitored via the biophoton output of an individual.

References

Bischof, M. (2005). Biophotons – The light in our cells. Journal of Optometric Phototherapy.

Cifra, M., Pospisil, P. (2014). Ultra-weak photon emission from biological samples: definition, mechanisms, properties, detection and applications. Journal of Photochemistry and Photobiology B: Biology.

Creath, K., Schwartz, G. E. (2005). Biophoton interaction in biological systems: evidence of photonic info-energy transfer? Proceedings of SPIE; 6285: 338-347.

Cohen, S., Popp, F. A. (1997). Biophoton emission of the human body. Journal of Photochemistry and Photobiology B: Biology; 40: 187-189.

Compton, A. H. (1923). A quantum theory of the scattering of X-rays by light elements. Physical Review; 21: 483-502.

Dotta, B. T., Buckner, C. A., Cameron, D., Lafrenie, R. M., Persinger, M. A. (2011). Biophoton emissions from cell cultures: biochemical evidence for the plasma membrane as the primary source. General Physiology and Biophysics; 30: 301-309.

Edwards R., Ibison, M. C., Jessel-Kenyon, J., Taylor, R. B. (1989). Light emission from the human body. Complementary Medical Research; 3: 16-19.

Einstein, A. (1905). Zur Elecktrodynamik bewegter Korper [On the electrodynamics of moving bodies]. Annalen der Physik; 322(10): 891-921.

Goldhaber, A. S., Nieto, M. M. Photon and graviton mass limits. Reviews of Modern Physics; 82: 939-979.

Jung, H., Yang, J., Woo, W., Choi, C., Yang J., Soh, K. (2004). Year-long biophoton measures: normalized frequency count analysis and seasonal dependency. Journal of Photochemistry and Photobiology B: Biology; 78: 149-154.

Maxwell, J. C. (1861). On physical lines of force. Philosophical Magazine; 21(139): 161-175.

Maxwell, J. C. (1864). A dynamical theory of the electromagnetic field. Philosophical Transactions of the Royal Society of London; 155: 459-512.

Michelson, A. A., Morley, E. W. (1887). On the relative motion of the Earth and the luminiferous ether. American Journal of Science. 34, 333–345.

Planck, M. (1901). Ueber die Elementarquanta der Materie und der Elektricität [On the elementary quantum of matter and electricity]. Annalen der Physik; 309(3): 564-566.

Popp, F. A. (1979). Electromagnetic bioinformation. New York: Urban and Schwarzenberg.

Popp, F. A., Gu, Q., Li, K. (1994). Biophoton emission: experimental background and theoretical approaches. Modern Physics Letters B; 8: 1269 – 1296.

Popp, F. A. (2009). Cancer growth and its inhibition in terms of coherence. Electromagnetic Biology and Medicine; 28: 53-60.

Rahnama, M., Tuszynski, J., Bokkon, I., Cifra, M., Sardar, P., Salari, V. (2011). Emission of mitochondrial biophotons and their effect on electrical activity of membrane via microtubules. Journal of Integrative Neuroscience; 10(1): 65-88.

Takeda, M., Tanno, Y., Kobayashi, M., Usa, M., Ohuchi, N., Satomi, S., Inaba, H. (1998). A novel method of assessing carcinoma cell proliferation by biophoton emission. Cancer Letters; 127: 155-160.

Van Wijk, R., Van Wijk, E. P. A. (2005). An introduction to human biophoton emission. Forsch Komplementarmed Klass Naturheilkd; 12: 77-83.

CHAPTER TWO

REPEATED MEASUREMENT OF BIOPHOTON EMISSION FROM TWO HUMANS OVER A FIVE-MONTH PERIOD: IMPLICATIONS FOR INDIVIDUAL VARIATION

Abstract

Living organisms emit photons at a low intensity level, as this emission is linked to metabolic processes at a cellular level. These photons are often referred to as biophotons. Human biophoton emission is a relatively novel field of study, with implications for the medical field. We repeatedly measured the biophoton emission from two male participants 138 times over a five-month period. Photon emission was recorded using four photomultiplier tubes in a specially-constructed dark experimental chamber. A discrimination function was able to correctly classify the two participants with 100% accuracy during the first month of the experiment. This discrimination function relied on the spectral characteristics of the photon emission, with the 0Hz to 3Hz frequency range as one of the key variables. Average photon counts were not a significant variable in the discrimination function. The ability to distinguish the two individuals was lost after the first month of the experiment. A possible explanation for the loss of discrimination is the homogenization of the experimental space. Implications for the use of human biophoton emission as a diagnostic tool are discussed.

Introduction

Human biophoton emission is a relatively novel area of experimentation, with the earliest reported studies in the mid-1970's. These studies were complicated by the noise produced by early models of photomultiplier tubes (PMTs), and had contradictory results. Two studies in 1989 and 1990 by Edwards and colleagues, systematically observed the UPE emitted by the hand every 1.5h, for a total of 28h; they also observed UPE emitted from other regions, including, the forehead, abdomen, lower back, and chest (Van Wijk, 2005). They found the level of UPE was increased in the hand and forehead regions compared to the chest, abdomen, and lower back (Edwards *et al*., 1989). Popp and his colleagues, notably Cohen, also conducted measurements on humans in the late 1990's and early 2000's. They were able to obtain information concerning the frequencies present within the UPE, significant light-right asymmetries, the presence of a 24h cycle, and a non-local effect (Bischof, 2005).

One of the main benefits of biophoton research in humans is its application as a diagnostic tool. The underlying mechanism to explain biophoton emission is metabolic activity, specifically oxidative processes (Cifra & Pospisil, 2014). This may be linked to the activity in the plasma membrane (Dotta *et al*., 2011). In diseased states, regular metabolic activity is often altered, typically increased compared to normal. This includes cancerous tissues and inflammatory responses. It has been demonstrated that increased metabolic activity in cell cultures led to significant increases in biophoton emission (Takeda *et al*., 1998). Dotta *et al*. found a significant increase in spectral power between malignant B16-BL6 and non-malignant HEK cell cultures (2011).

Many studies concerning human biophoton emission look specifically at the hands, an area with high photon counts in comparison to other body regions (Van Wijk, 2005). Jung *et al.*

performed a year long study of human biophoton emission, with the left and right hands as the region of interest (2005). Cohen and Popp measured an individual over a 9-month period, but only focused on the hands and forehead (1997). In these studies, the PMT was positioned directly above the region of interest. This limits the generalization of the results to the rest of the body. The use of biophoton emission as a diagnostic tool would ostensibly require global measurement before localized methodology could be used. There has not been a large research effort directed at the measurement of whole body biophoton emission. The few studies that have been conducted do not focus on repeated measurements of the same individual.

Methodology has a noticeable impact on the collection of data, due to the low intensity and diffuse nature of biophoton emission. The majority of biophoton activity is absorbed by the body (Rahnama *et al*., 2011). This leaves only a small amount that can escape and be observed. It is estimated that the external biophoton emission is two orders of magnitude lower than endogenous activity (Rahnama *et al.*, 2011). Conditions such as temperature and humidity can change daily, and others like the technical aspects of a PMT or the distance between the PMT and the subject vary between laboratories. This makes the comparison of data difficult at best. One factor that is not well understood is why there is a slow decay of photon counts at the onset of recording. This effect is reportedly related to exposure to artificial light sources outside of the measurement area. Termed “delayed luminescence”, this is thought to be a contamination of the photon activity, and that requires longer measurement periods to collect reliable and consistent photon counts (Van Wijk, 2005). Understanding this effect is essential for accurate comparisons of biophoton activity.

This experiment aimed to conduct a long-term, repeated measurement of two participants. Data obtained could then be used to identify trends or patterns within each participant, or be used to discriminate between them. Comparisons between human and baseline conditions may help in

the identification of a human biophoton “signature”. All of these aims can also be applied over time, to observe if biophoton emission from an individual shifts over time, or remains constant.

Methods

One hundred and thirty-eight biophoton measures from two healthy male participants, aged 22 and 26, were taken over a period of 156 days from February 10, 2017, to July 15, 2017. Biophoton measurements were taken once every 24 period.

Each daily measurement was performed using the same measurement chamber. The measurement chamber is a large wooden box, with dimensions 1.7m in height, 1.27m in length, and 1.32m in width. The chamber is painted black to minimize the amount of external photon exposure from entering while the chamber is closed. Additionally, the entire outside surface is covered with black towels to minimize contamination from external emissions. Inside the chamber, a plastic chair is placed directly in the centre for a participant to sit on during measurement. Four photomultiplier tubes (PMTs) are placed within the chamber. There is a PMT on each of the walls of the chamber, excluding the wall with the door, designated F-PMT, B-PMT, H-PMT, and one placed on the ceiling, designated Z-PMT. F-PMT is placed on the front wall; B-PMT is placed on the back wall; H-PMT is placed on the side wall opposite the door of the chamber; Z-PMT is placed on the ceiling directly above the participant. The three PMTs on the walls of the chamber are all placed in the midpoint of the wall horizontally, and approximately 0.15m from the center of the chamber.

First, a baseline recording of the empty measurement chamber was taken. This measurement was 4 minutes in duration at a sampling rate of 50Hz. The measurement chamber was not opened prior to this recording. This provided a baseline recording each day. After the baseline recording, one of the participants would enter the measurement chamber. Once the chamber was closed, there was a one-minute delay before the measurement began. This was to allow the biophoton measurement to estimate and approach a consistent output. After this one-

minute delay, a four-minute measurement was taken at a sampling rate of 50Hz. Once the measurement was completed, the participant would exit the chamber. The second participant would then enter the chamber, and the same process as described above was repeated. The order in which the two participants were measured was alternated each day. After both participants were measured, a fourth recording was taken. The process described above was repeated except that a white polystyrene foam box was used to substitute for the position of the participant. This measurement was taken to observe how the photon emission changes without a biological system inside the chamber. Other pertinent data, such as any odd patterns, time of recording, etc. were noted by the experimenters for each experimental day.

Once the measurements were completed, data was extracted using Microsoft Excel. The data from each PMT was then subjected to a spectral analysis with the software program IBM SPSS. Data was truncated into average spectral power bins with a width of 1Hz and 0.1Hz, for further statistical analysis. These bins were used in addition to the average photon count per second. Means and standard deviations were used for both of these variables. Standardized mean photon counts were calculated by computing the average mean photon count and standard deviation of the mean photon count from all experimental days, and computing z-scores based on those values for each experimental day. This was done separately for each PMT.

Results

A total of 136 valid days of measurement were used in the analysis, after removing outliers and days where no recording took place.

A discriminant analysis was performed to differentiate between the post-measurement and participant conditions. Spectral frequency bins, as well as mean intensities and standard deviations were included as possible variables. The discriminant function was able to correctly identify 90.2% of all cases ($\Lambda = .455$, $\chi_{(4)} = 319.273$, $p < .001$). Four main factors loaded in this function, shown in Table 2-1 below.

***Table 2-1*. The main factors that loaded onto the discriminant function, and their standardized canonical coefficients.**

Variable	Standardized Canonical Discriminant Function Coefficient
B-PMT mean intensity	-1.687
H-PMT mean intensity	-0.176
F-PMT standard deviation	1.033
B-PMT standard deviation	1.334

Figure 2-1 shows the scores of the four variables between human and baseline conditions.

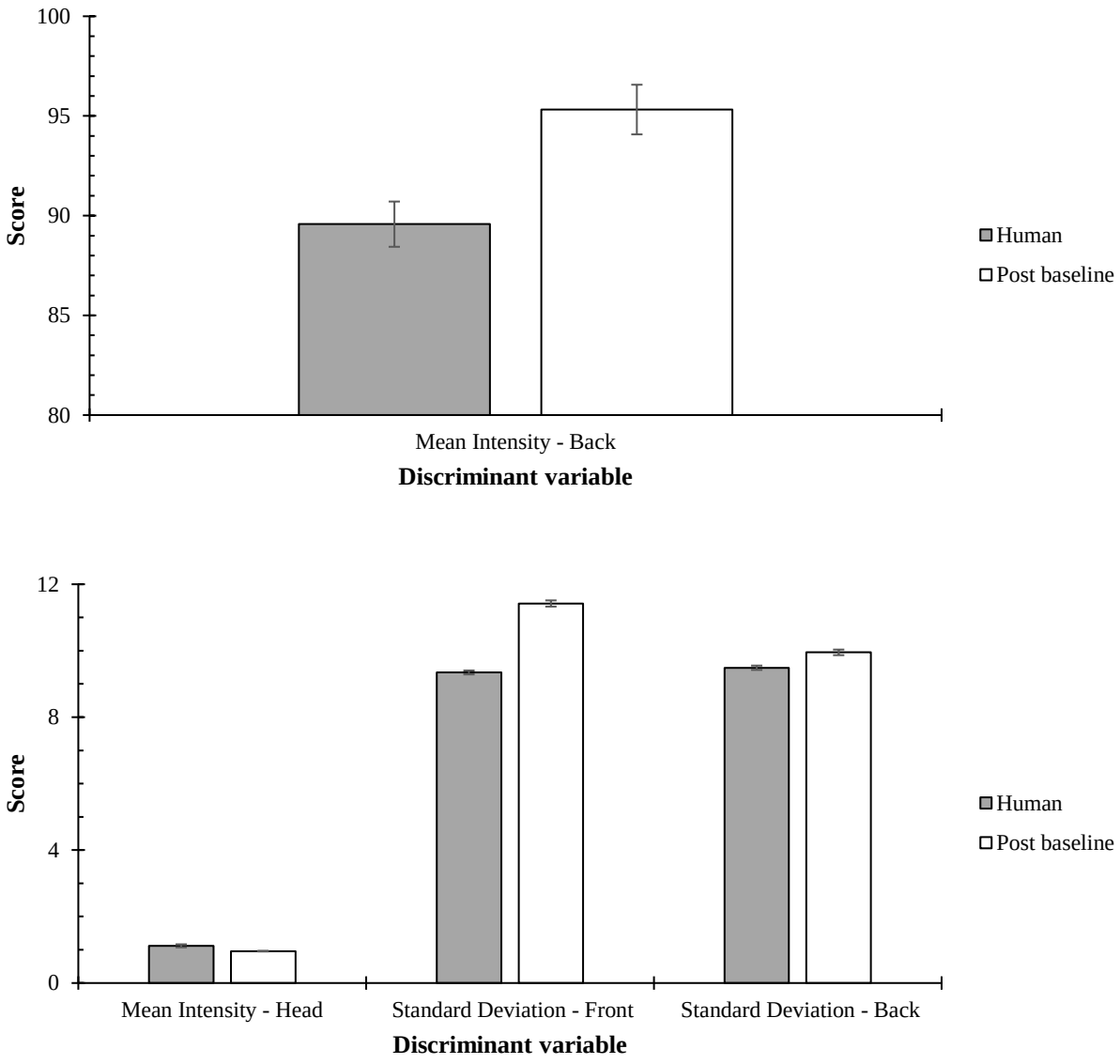

***Figure 2-1*. Comparison of the four variables that loaded on the discriminant function. The mean intensity of B-PMT has been included on a separate axis for scale. Error bars represent standard error of the mean, n = 136.**

There were no significant differences between the mean intensity of each PMT by participant (Front: $F_{(1,272)} = 0.678$, $p = .411$; Back: $F_{(1,272)} = 0.330$, $p = .566$; Zed: $F_{(1,272)} = 0.198$, $p = .565$; Head: $F_{(1,272)} = 0.009$, $p = .923$). These results are shown in Figure 2-2 below.

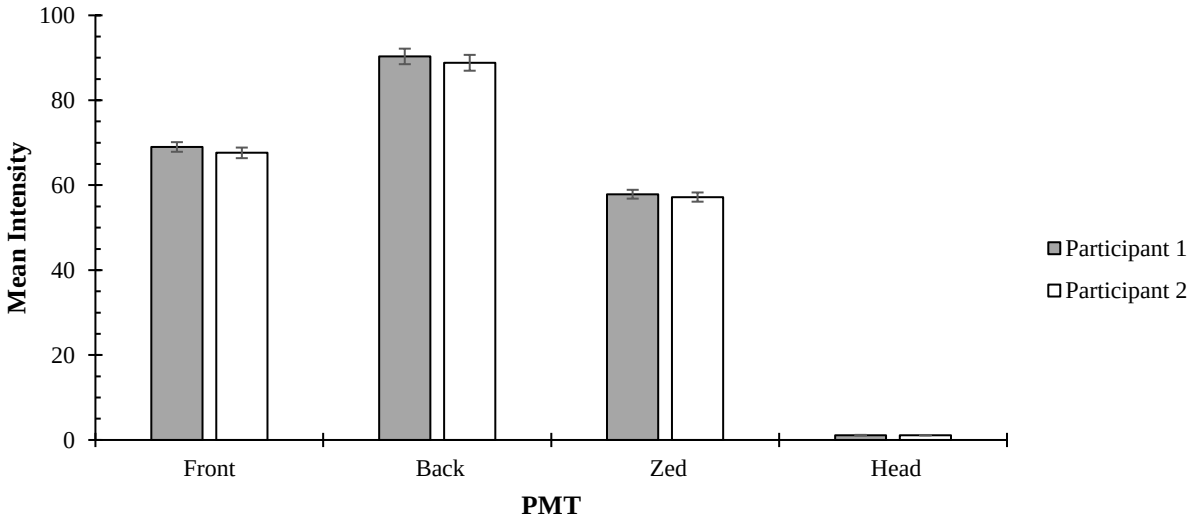

***Figure 2-2*. Comparison of mean intensities of photon recordings for each PMT across participants. None of the PMTs were significantly different between participants (α = .05). Error bars represent standard error of the mean, n = 136.**

A second discriminant analysis was performed to differentiate between participants. This analysis used the same variables as the first discriminant analysis. No function was able to discriminate between participants across all 136 cases. As a follow up, multiple discriminant analyses were performed on each month of recording. Only the first month of recording produced a significant discriminant function. This function was able to correctly identify 100% of all cases (Λ = .191, $\chi_{(6)}$ = 54.595, p < .001). The variables that loaded onto this discriminant function are shown below in Table 2-2.

***Table 2-2*. The main factors that loaded onto the participant discriminant function, and their standardized canonical coefficients. The delta frequency bin has a frequency width of 0-3Hz; the alpha frequency bin has a frequency width of 7-10Hz.**

Variable	Standardized Canonical Discriminant Function Coefficient
F-PMT standard deviation	1.984
B-PMT standard deviation	-1.315
H-PMT standard deviation	1.020
Delta spectral frequency bin	-1.098
Alpha spectral frequency bin	-0.504
B-PMT coefficient of variation	0.759

Figure 2-3 shows the scores of each participant and their discriminant score.

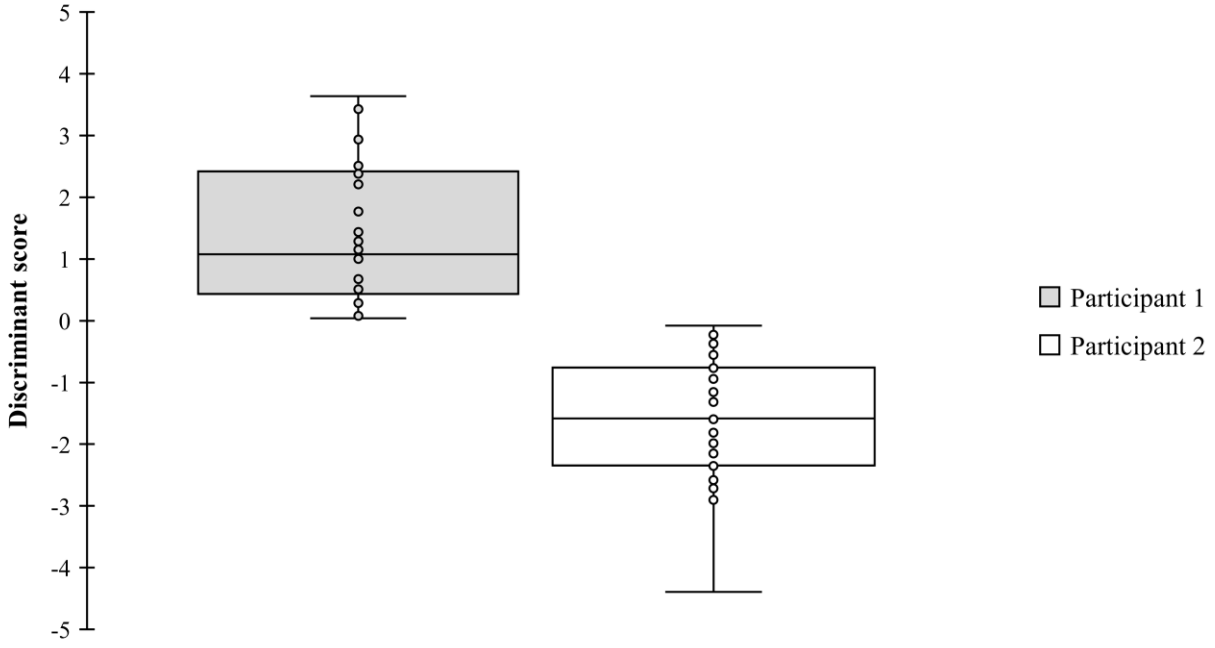

***Figure 2-3*. Box plot of the participant discriminant results. Please note that there is no overlap between the minimum of Participant 1 and the maximum of Participant 2.**

In order to determine if the discriminant was spurious, a discriminant analysis was performed for each week of the experiment. The prediction accuracy was averaged for each month (every four weeks). If the analysis was unable to produce a discriminant function, an accuracy score of 0 was assigned to that week. The results of this procedure are shown in Figure 2-4.

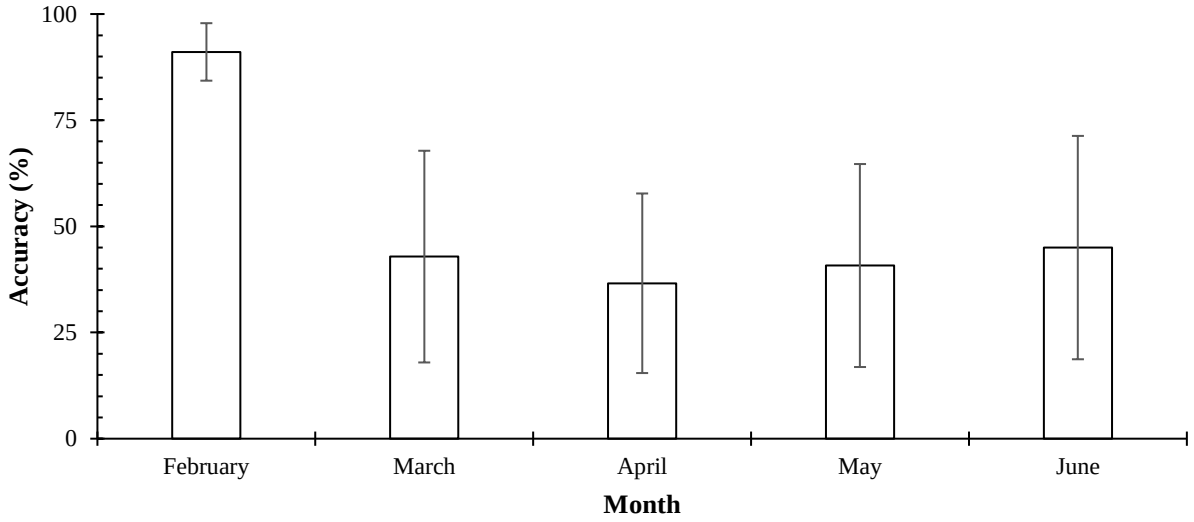

***Figure 2-4*. Accuracy of discriminant functions over time, for differentiation of participants. The accuracy of discriminant functions is highest in the first month of the experiment, after which the accuracy drops off sharply for the remainder. Error bars represent standard error of the mean, n=4.**

The mean photon counts for each PMT by condition are shown in greater detail in Figure 2-5 below. A one-way ANOVA revealed there was a significant effect between the three conditions (Pre-baseline, Human, Post-baseline) for each PMT (F-PMT: $F_{(2, 541)} = 200.357$, $p < .001$, $\eta^2 = .426$; B-PMT: $F_{(2, 541)} = 40.852$, $p < .001$, $\eta^2 = .131$; Z-PMT: $F_{(2, 541)} = 31.433$, $p < .001$, $\eta^2 = .104$; H-PMT: $F_{(2, 541)} = 60.686$, $p < .001$, $\eta^2 = .183$). Post-hoc tests using Tukey HSD revealed that each condition was significantly different from one another ($\alpha = .05$), in each PMT. The post-baseline condition had the highest mean photon counts, followed by the human condition; however, this trend was not seen with H-PMT's mean photon counts, where the post-baseline

condition was lower than the human condition. The pre-baseline condition had the lowest mean photon counts in all PMTs. The standardized mean photon counts for the participants were not significantly different from one another in any of the PMTs, so they were grouped together as the human condition.

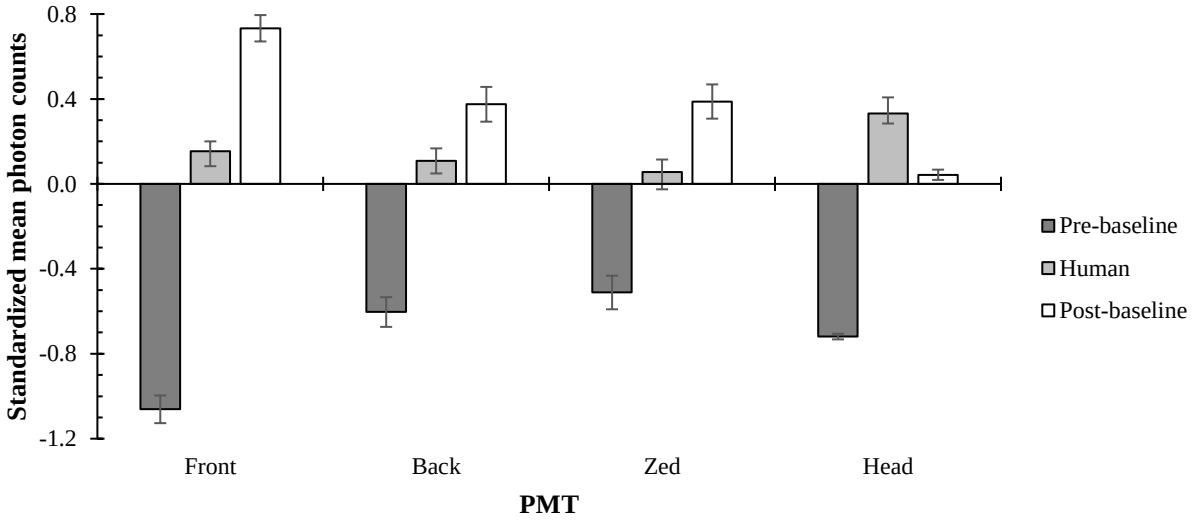

***Figure 2-5*. Comparison of the standardized mean photon counts per condition from each PMT. Mean photon counts were standardized within each PMT. The pre-baseline condition has the lowest photon counts in all PMTs. In H-PMT, the human condition was higher than the post-baseline condition, which was the opposite of the other three PMTs. Both participants were grouped together, as they were not significantly different from each other. All conditions were significantly different. Error bars represent standard error of the mean, n = 136.**

Discussion

Mean photon counts are perhaps the easiest statistic to observe directly from PMT recordings. Given that four PMTs were used throughout the experiment, this leaves four mean counts to compare for each recording session. Direct comparisons are potentially possible for three of the four PMTs (F-PMT, B-PMT, Z-PMT), but as the H-PMT is a different model of PMT from the previous three, direct comparisons are not possible. The magnitude of the mean photon counts from the two models of PMTs used can differ by up to a power of 10. Thus, standardization within each PMT is essential if any comparisons are to be made.

Unfortunately, due to the nature of the experimental process, mean photon counts are still not an optimal variable for discrimination between human conditions. They may not even be a discriminating factor between human and baseline conditions. Mean photon counts are useful for differentiation between conditions where the experimental chamber was exposed to outside light sources, or if the experimental chamber remained closed. The methods used for this study involved the recording of one "pre-baseline" measurement and one "post-baseline" measurement per recording session; this was done to observe if there was a noticeable difference in the mean photon counts from simply allowing outside light to enter the chamber. This phenomenon is known as delayed luminescence, and has been described previously (Popp & Yan, 2002).

Light contamination seems to have the largest effect on mean photon counts at the start of the recording. As this light contamination dissipates and the dark condition of the experimental chamber returns to normal levels, the photon count decreases. An example of this can be seen in Figure 2-1. This gradual decrease was not observed in the pre-measurement baseline condition where the experimental chamber was not opened before recording. Due to light contamination, the

mean photon counts were significantly higher in the post-measurement baseline condition were the experimental chamber was opened compared to if the chamber was not opened.

A discriminant analysis could not successfully compute a model to differentiate between human and baseline conditions. Variables for this analysis included mean photon counts, spectral scores, and variance statistics including the standard deviation of photon counts and the coefficient of variation of photon counts. If the pre-measurement baseline condition (with no light contamination) was excluded from the analysis, the discriminant analysis was able to differentiate between human and post-measurement baseline conditions with a 90% classification accuracy. If light contamination is present in the collection of data, it seems that its inclusion or exclusion is enough to impact findings. Popp and Yan have previously shown that a hyperbolic equation can be used to mimic the decay of photon counts, and that there are differences in these oscillations between healthy and cancerous tissue (2002). Those findings relate to plant tissue and may not be relevant for other biological systems.

Mean photon counts and light contamination are important for discrimination between human and baseline conditions. For discrimination between human participants, mean photon counts are less important. Figure 2-2 shows a comparison of mean photon counts between participants. There were no significant differences in the mean photon counts between participants.

Mean photon counts are not the only data that can be obtained from PMT recordings. A spectral analysis can also be performed to discover trends within the data. A spectral analysis allows for the observation of repetitive cycles, or frequencies, that may not be evident in the intensity measures. With respect to photon measurements, a spectral analysis would convey information about the patterns in the photons that interact with the PMT, if any. For example, this analysis could indicate if there was a 20Hz pattern present, or if about one photon was reliably

emitted every 0.5ms. This may be related to the metabolic rate or some intrinsic property with respect to the tissue.

The discriminant analysis between participants revealed that were two variables derived from the spectral analyses of the recorded data. These variables showed that photons emitted within the delta frequency bin and the alpha frequency bin could distinguish participants. These bins represent frequencies of 0-3Hz and 7-10Hz, respectively. The frequency bins are calculated from the average of thousands of smaller frequencies obtained from the spectral analyses performed on each recording. Each measurement was composed of 12000 points; the spectral analysis leaves half of that, 6000 points. The largest frequency that can be measured by a spectral analysis is half of the sampling rate. In this experiment, the sampling rate was 50Hz; thus, the limit of the spectral analysis is 25Hz. This means that each point from the spectral analysis is representative of a frequency width of $4.1\overline{7}$mHz (25Hz / 6000 points = $4.1\overline{7} \times 10^{-3}$ Hz/point). Thus, the 3Hz frequency bins are the average of 720 points.

The condensation of the frequency bins is not unique to this analysis, and has been previously performed with electroencephalograph results. This may help to justify the generalization of the results found in this experiment with other biophoton experiments. Discrimination analyses between living and non-living systems, and between living systems of different states (healthy vs. cancer), have been performed previously; however, little to no work has been performed to discriminate or identify differences between individuals or to determine if the same individual is consistent over time.

The inclusion of thousands of variables would not be an efficient way to evaluate possible differences between individuals in this novel branch of experimentation. Simplification of the data prior to analysis allows for more generalizable results within and between participants. While the

intricacies of the data may be lost this way, the thousands of variables will lead to spurious effects based on confounding problems related to multiple testing. Condensation of these variables allows for the main effects, if any, to remain observable while the efficacy of the statistical approach is maintained.

The interpretation of the frequency bins, in a biophoton experiment, is novel. The discriminant analysis that was able to differentiate between individuals for February 2017 only revealed two spectral variables that were significant: the delta (0-3Hz) frequency bin and the alpha (7-10Hz) frequency bin. It is beyond the scope of this experiment to determine what the specific biological origins of these frequency bins may be. Rather, general hypotheses will be examined.

Delta frequencies may be associated with the long-term metabolic activity of the participant. Biophoton emissions are a consequence of the metabolic processes associated with life. There exists a myriad of factors that influence what an individual's specific metabolic rate may be at any given moment, but there must be a constant, slow supply of various molecules to ensure continued biological function. This underlying metabolic activity may be the source of the delta frequencies observed. Prasad and colleagues have discussed biophoton emission as possible mediator of intercellular communication (Prasad *et al*., 2014); therefore, these frequencies may also be related to intercellular communication.

Alpha frequencies may be associated with Schumann resonances. Schumann resonances are frequencies in the 7-8Hz range that are caused by the interaction between global lightning strikes in the ionosphere and Earth's surface. The resonant frequencies can be shifted slightly by interactions with seismic energy, the height of the ionosphere, and the time of year. Schumann resonances have been correlated with electroencephalograph activity in humans (Saroka & Persinger, 2014). There may exist a correlation between Schumann resonance, brain activity, and

biophoton activity that could explain the source of the differences in the observed alpha frequency bin.

Though a successful discriminant function was found for the month of February, no significant functions were able to differentiate between participants for any of the other four months (March – June), or across the entire experiment. One potential explanation for this may be a spatial light pollution of the experimental chamber. Just as the introduction of outside light into the experimental chamber produced notable changes in recorded variables measured on each day over time, the repeated presence of the participants inside the chamber may have produced changes in the space of recording.

Some support for this claim is present from the data collected from the mean photon counts across each of the PMTs per condition. In the F-PMT, B-PMT, and Z-PMT, mean photon counts trend with the order of measurement, excluding the measurements of the human participants which were consistently similar. This means that the post-baseline condition had a higher mean photon count than the two human conditions, which seems contradictory to previous research. Crucially, this trend is not observed in the H-PMT. In the H-PMT recordings, post-baseline mean photon counts are higher than the pre-baseline, but not higher than the participants' mean photon counts. The H-PMT recordings also seem to be the least sensitive to light contamination, as seen in the photon counts over time.

Similar to the way the participants biophoton signature contaminated the post-baseline measurement, they may have also contaminated the experimental space. This would lead to a homogenization of the environment within the experimental chamber as the participants continued to contaminate the light-sensitive environment. As this increase in contamination in the participants contributed to background processes and interfered, interfaced, or overlapped with the

biophoton output measured from the experimental space, they may have obscured the necessary information for discrimination.

Another possible explanation for the lack of significant discrimination may simply be due to the frequency bin sizes. The frequency bins used in the analysis were averages of 720 points. It is possible that these bins are simply too large and mask details that are required for the differentiation of individual participants. An increase in the sample size would be necessary for the inclusion of more variables. While 136 distinct days of measurement is a good start, more data is needed for that level of statistical testing.

This experiment was able to achieve some of its aims. Discrimination between human and baseline conditions was successful. Special care must be taken when working with light contamination. Discrimination between human participants was partially successful in one month of the experiment. A possible explanation for this inconsistency was the spatial contamination of the experimental chamber. Individual differences in slow rhythm metabolism may be a factor in the significance of the delta frequency band that allowed for the differentiation of the two participants.

The data that was measured by this experiment will be useful for future discrimination experiments. Taking proper and regular baselines is essential for successful interpretation. Spectral analyses can focus on the delta (0-3Hz) and alpha (7-10Hz) frequency ranges. Environmental factors, including humidity and temperature, must also be considered. Diagnostic information requires a healthy baseline for comparison. Ensuring accurate readings is paramount for the use of biophoton emission for the potential detection of cancerous tissues.

References

Bischof, M. (2005). Biophotons – The light in our cells. Journal of Optometric Phototherapy.

Cifra, M., Pospisil, P. (2014). Ultra-weak photon emission from biological samples: definition, mechanisms, properties, detection and applications. Journal of Photochemistry and Photobiology B: Biology.

Cohen, S., Popp, F. A. (1997). Biophoton emission of the human body. Journal of Photochemistry and Photobiology B: Biology; 40: 187-189.

Dotta, B. T., Buckner, C. A., Cameron, D., Lafrenie, R. M., Persinger, M. A. (2011). Biophoton emissions from cell cultures: biochemical evidence for the plasma membrane as the primary source. General Physiology and Biophysics; 30: 301-309.

Edwards R., Ibison, M. C., Jessel-Kenyon, J., Taylor, R. B. (1989). Light emission from the human body. Complementary Medical Research; 3: 16-19.

Jung, H., Yang, J., Woo, W., Choi, C., Yang J., Soh, K. (2004). Year-long biophoton measures: normalized frequency count analysis and seasonal dependency. Journal of Photochemistry and Photobiology B: Biology; 78: 149-154.

Prasad, A., Rossi, C., Lamponi, S., Pospisil, P., Foletti, A. (2014). New perspective in cell communication: potential role of ultra-weak photon emission. Journal of Photochemistry and Photobiology B: Biology; 139: 47-53.

Popp, F. A., Yan, Y. (2002). Delayed luminescence of biological systems in terms of coherent states. Physics Letters A; 293: 93-97.

Rahnama, M., Tuszynski, J., Bokkon, I., Cifra, M., Sardar, P., Salari, V. (2011). Emission of mitochondrial biophotons and their effect on electrical activity of membrane via microtubules. Journal of Integrative Neuroscience; 10(1): 65-88.

Saroka, K. S., Persinger, M. A. (2014). Quantitative evidence for direct effects between Earth-ionosphere Schumann resonances and human cerebral cortical activity. International Letters of Chemistry, Physics, and Astronomy; 20(2): 166-194.

Takeda, M., Tanno, Y., Kobayashi, M., Usa, M., Ohuchi, N., Satomi, S., Inaba, H. (1998). A novel method of assessing carcinoma cell proliferation by biophoton emission. Cancer Letters; 127: 155-160.

Van Wijk, R., Van Wijk, E. P. A. (2005). An introduction to human biophoton emission. Forsch Komplementarmed Klass Naturheilkd; 12: 77-83.

CHAPTER THREE

IMPACT OF NON-ORGANIC MATERIAL ON BIOPHOTON EMISSION FROM B16-BL6 AND C2C12 CELL LINES: DISCRIMINATION BETWEEN HEALTHY AND CANCEROUS CELL LINES

Abstract

Metabolic activity is correlated with biophoton emission. Cancerous and non-cancerous cell types express different rates of metabolic activity. This allows for the potential discrimination of these cell lines based on their biophoton emission. “Healthy” non-cancerous and cancerous cell lines were allowed to grow and approximately 10^6 cultured cells and were placed within a nutrient agar moulds of different volumes. The biophoton activity of these cultures were measured for one minute at a 50Hz sampling rate. No significant differences were found as a function of agar mould volume. B16-BL6 cell cultures had significantly higher mean photon counts than controls, and produced different spectral power densities in multiple frequency ranges. B16-BL6 cell cultures and C2C12 cell cultures had significantly different spectral power densities in the 19.50Hz to 19.99Hz frequency range. Implications for future discrimination studies are discussed.

Introduction

Ultraweak photon radiation from a biological source, or biophoton emission, has been measured in cultures of animal and plant tissue. The origin of this energy has been proposed to originate from metabolic reactions within the cell. The oxidation of free radicals acts as one of the primary sources of biophoton emission (Dotta *et al*., 2011). As such, changes in metabolic activity should alter some quality of biophoton emission. This has been demonstrated via temperature dependence on other metabolic reactions such as glycolysis (Popp, 2009). Differences in metabolism as a function of cell type may also be observable.

A potential application of the ability to differentiate between cell type would be to develop a diagnostic test for cancerous cells. Biophoton emission presents a non-invasive measurement of internal biological activity. Takeda *et al.* were able to demonstrate that the biophoton output of cancer cells was dependent on the size of the colony (1998). Later work performed by the same researchers was able to create images of tumors transplanted into mice with only biophoton emissions (Takeda *et al*., 2004).

One issue is the potential interference of surrounding non-malignant biological tissue. Characteristic activity of specific cell types will be necessary to accurately detect diseased tissue in an animal. In addition, the amount of biological tissue that may interfere can vary; for example, an epidermal melanoma would have a much lower amount of tissue within and around it compared to a breast cancer lipoma. Cancer growth has been described as an emergent phenomenon of the cell population (Popp, 2009). Understanding the potential interference that is a consequence of surrounding tissue may allow for increased detection.

To that end, the biophoton emission of cell cultures, including human and murine cell lines, were observed. The effect of an inert mass around each cell culture was modified by the placement of the cells within a nutrient agar mould of different volumes; greater volume of agar corresponds to a greater tissue depth. The aims of this experiment were to determine the impact of this artificial depth on the characteristics of biophoton emission, if any, and to differentiate between the observed cell lines.

Methods

Various cell lines including HSG (human submandibular gland cells), AsPC (human pancreatic cancer cells), MCF-7 (human breast cancer cells), B16-BL6 (murine malignant melanoma cells), C2C12 (murine muscle cells), NIH (murine mesenchymal cells), and 4T1 (murine breast cancer cells), were cultured in DMEM containing 10% fetal bovine serum and 1% antibiotics (Hyclone). Cells were separated using trypsin, counted, and 10^6 cells were suspended in 50µL Matrigel. This culture suspension was then placed within one of three containers that held a volume of 1% nutrient agar dependent on the size of the container. These were termed small (~50mL volume), medium (~100mL volume) and large (~500mL volume). A channel was created in the agar so that the cell culture was placed in the centre of the container, regardless of the size. Controls were performed using each of the containers containing only agar. Biophoton measurements were obtained within 24h after the culture was placed within the agar container. The container was then measured for one minute at a sampling rate of 50Hz within the experimental chamber. Within the chamber, four individual photomultiplier tubes (PMTs) were used to record biophoton activity. Baseline recordings were also performed. These were done by opening the door of the experimental chamber as if a container had been placed inside and then performing a normal measurement.

After recording, the data was imported into IMB SPSS for standardization, and statistical and spectral analyses. Mean photon counts were standardized with respect to each PMT to allow for comparison between PMTs.

Results

A one-way ANOVA was performed to compare the standardized mean photon counts of the seven cell conditions; B16-BL6, NIH3T3, HSG, MCF-7, 4T1, AsPC, and C2C12. No violations of homogeneity were found ($W_{(8,109)} = 1.123$, $p = .353$). No significant effect was found between cell lines ($F_{(8,109)} = 1.787$, $p = .087$). The mean photon counts are shown below in Figure 3-1.

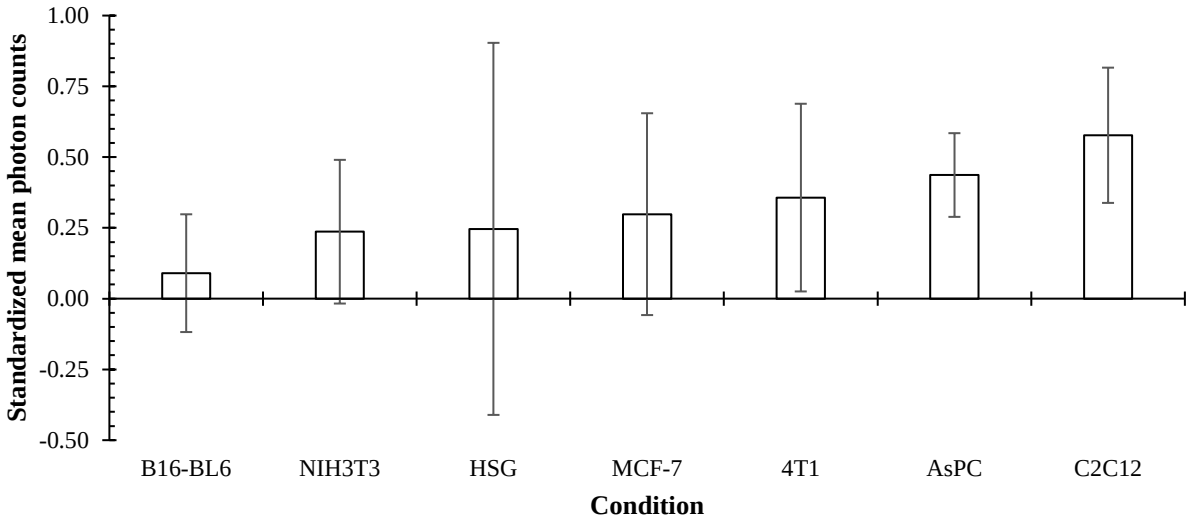

***Figure 3-1*. Comparison of standardized mean photon counts as a function of cell line. The sample size of each condition was 33, 13, 4, 3, 5, 10, and 10, respectively. There were no significant differences as a function of cell line. Error bars represent standard error of the mean.**

Due to low sample sizes, the majority of the results below use the B16-BL6 samples, as they were the cell line with the largest sample size of 33.

A one-way ANOVA was performed to compare the mean photon counts of the baseline, control, and B16-BL6 conditions. No violations of homogeneity were found ($W_{(2,69)} = 3.285$, $p = .593$). A significant main effect was found ($F_{(2,69)} = 3.285$, $p = .043$, $\eta^2 = .087$); post-hoc Tukey

HSD revealed the B16-BL6 condition was significantly higher than the control condition. Baseline conditions were not significantly different from either B16-BL6 or control conditions. This is shown in Figure 3-2 below.

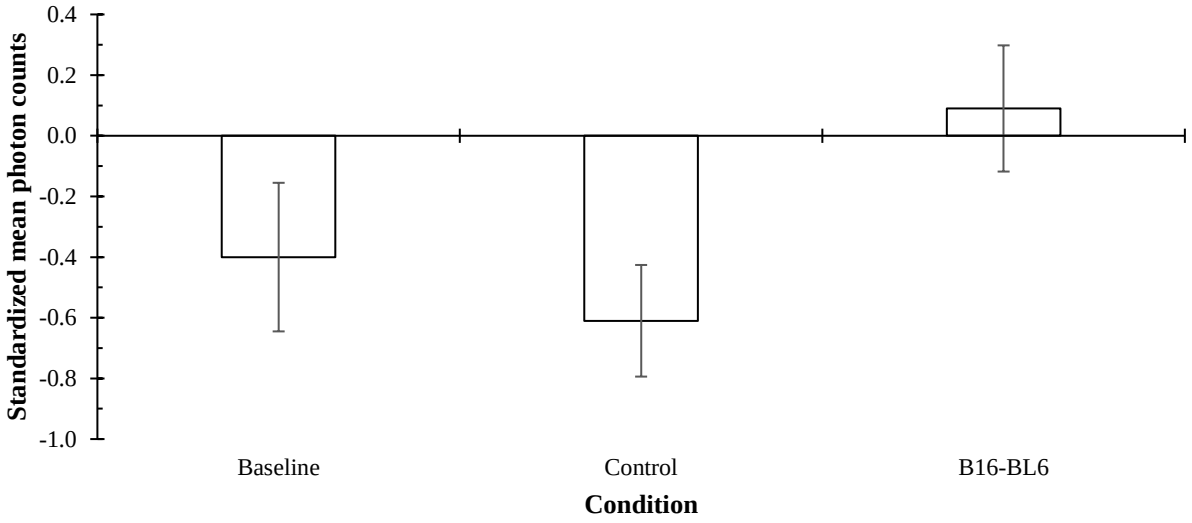

***Figure 3-2*. Comparison of the standardized mean photon counts between the baseline, control, and B16-BL6 conditions. The sample size of each condition was 5, 34, and 33, respectively. Control and B16-BL6 conditions were significantly different from each other; the baseline condition was not significantly different from either. Error bars represent standard error of the mean.**

Mean photon counts were compared as a function of sample size, including the control condition. This included all cell lines. A one-way ANOVA revealed a significant main effect (($F_{(3,146)} = 4.079$, $p = .008$, $\eta^2 = .078$); post-hoc Tukey's HSD revealed that the small, medium and large container conditions were significantly higher than the control conditions, but not significantly different from one another. These results are shown below in Figure 3-3.

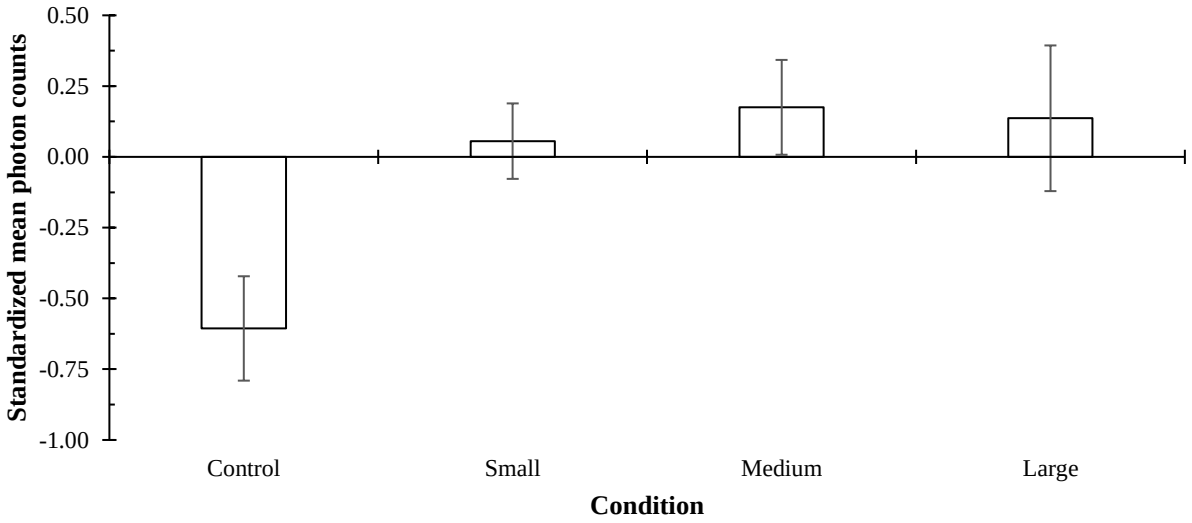

Figure 3-3. **Comparison of standardized mean photon counts as a function of container size. The sample size of each condition was 34, 54, 40, and 22, respectively. All size conditions were significantly higher than the control condition, but not significantly different from each other. Error bars represent standard error of the mean.**

Comparisons of spectral power density were also performed between control conditions and small, medium, and large container sizes. For this only B16-BL6 samples were used, since different cell lines have been shown to generate different spectral power densities. Homogeneity of variance was violated for all spectral power density comparisons as shown by Levene's test ($p < .05$); the Kruskal-Wallis test was used as a non-parametric substitute for the following procedures as noted.

The first frequency bin that was compared was the low frequency bin (0-0.9Hz). There was a significant difference between the four conditions ($\chi^2_{(3)} = 15.074$, $p = .002$, $\eta^2 = .203$); post-hoc homogenous subsets revealed that the control condition was significantly different from the B16-BL6 samples of all container sizes. There were no differences between the different sizes of container. The results are shown below in Figure 3-4.

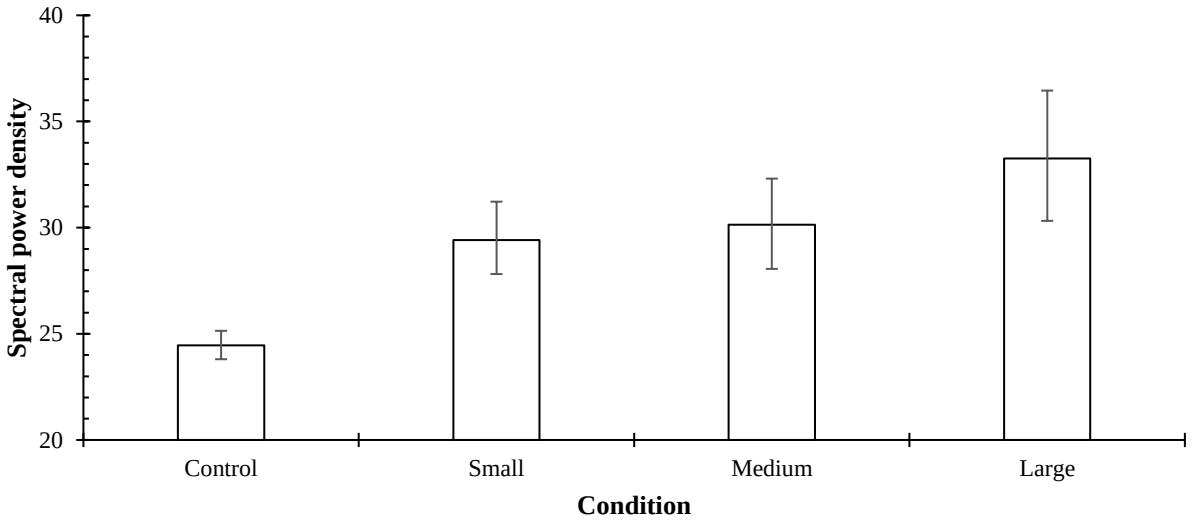

Figure 3-4. **Comparison of the spectral power density from the low frequency bin (0-0.9Hz) between B16 container sizes and control samples. Sample size for each group is 34, 19, 9, and 6, respectively. The small, medium, and large spectral power density was significantly greater than the control condition; they were not significantly different from each other. Error bars represent standard error of the mean.**

The above procedure was repeated for the remaining frequency bins: delta (1.0 – 4.9Hz), theta (5.0 – 8.9Hz), alpha (9.0 – 12.9Hz), low beta (13.0 – 16.9Hz), mid beta (17.0 – 20.9Hz) and high beta (21.0 – 25.0Hz). The relevant statistics are shown in Table 3-1 below.

Table 3-1. **Comparison of the Kruskal-Wallis tests comparing spectral power density between container sizes, per frequency bin. The degrees of freedom for all tests was 3.**

Frequency bin	Test statistic (χ)	Probability (p)	Effect size (η^2)
Delta	14.366	.002	.422
Theta	20.227	< .001	.507
Alpha	20.012	< .001	.504
Beta, low	13.785	.003	.412
Beta, mid	22.107	< .001	.529
Beta, high	18.428	< .001	.484

The post-hoc tests for each Kruskal-Wallis analysis were consistent; the control condition was always significantly different from the small, medium, and large container sizes, and the cell container sizes were not significantly different from each other. The key difference between the spectral power density of the low frequency bin and the six other frequency bins was that the trend reversed. In the low frequency bin, the cell conditions had a greater spectral power density than the control condition while the opposite was true for the other six frequency bins. As an example, the mid beta frequency bin is shown below in Figure 3-5.

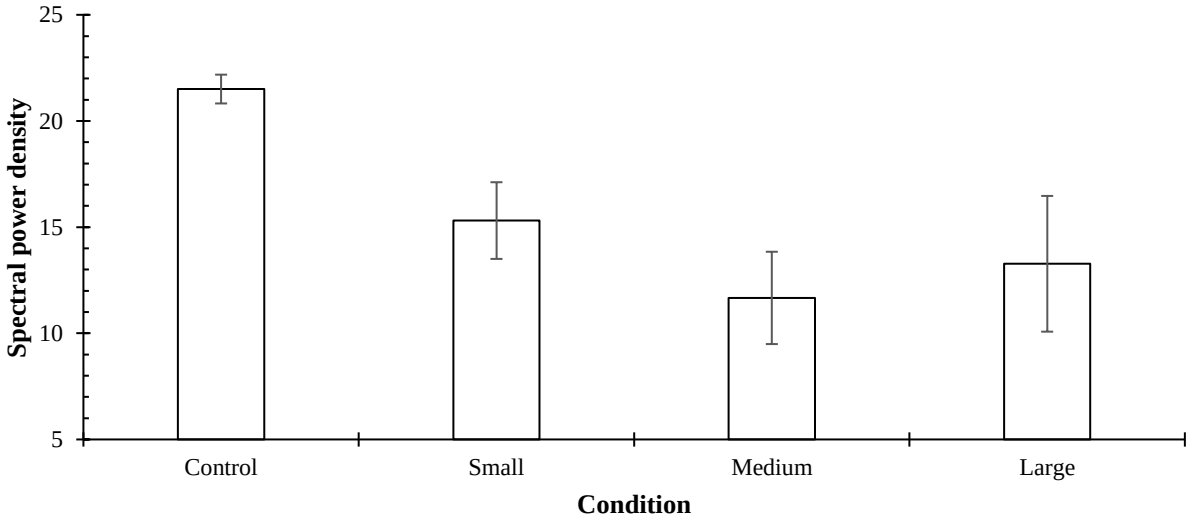

***Figure 3-5*. Comparison of the spectral power density from the mid beta frequency bin (17.0 - 20.9Hz) between B16-BL6 container sizes and control samples. Sample size for each group is 34, 19, 9, and 6, respectively. The small, medium, and large spectral power density was significantly lower than the control condition; they were not significantly different from each other. Error bars represent standard error of the mean.**

Next, the spectral power density of the control condition and the B16-BL6 condition was compared with the baseline condition. B16-BL6 cell samples were grouped together, regardless of container size. Homogeneity of variance was not met ($p < .05$), and non-parametric Kruskal-Wallis

tests were used to compare means across frequency bins. The results from these tests are shown in Table 3-2 below.

***Table 3-2*. Comparison of the Kruskal-Wallis tests comparing spectral power density between baseline, control, and B16-BL6 conditions, per frequency bin. Degrees of freedom for all tests was 2.**

Frequency bin	Test statistic (χ)	Probability (p)	Effect size (η^2)
Low frequency	17.155	< .001	.342
Delta	16.300	< .001	.331
Theta	20.705	< .001	.386
Alpha	22.013	< .001	.400
Beta, low	12.177	.002	.270
Beta, mid	21.918	< .001	.399
Beta, high	21.090	< .001	.390

Post-hoc tests revealed that the baseline condition was not significantly different from the control condition in all frequency bins. The baseline condition was significantly different from the B16-BL6 condition, with the exception of the low beta frequency bin. In this bin, the cell and control conditions were still significantly different. The trends were dependent on the frequency bin, similar to Figure 3-4 and Figure 3-5. Figure 3-6A shows the spectral power density in the low frequency bin; Figure 3-6B shows the spectral power density in the alpha frequency bin.

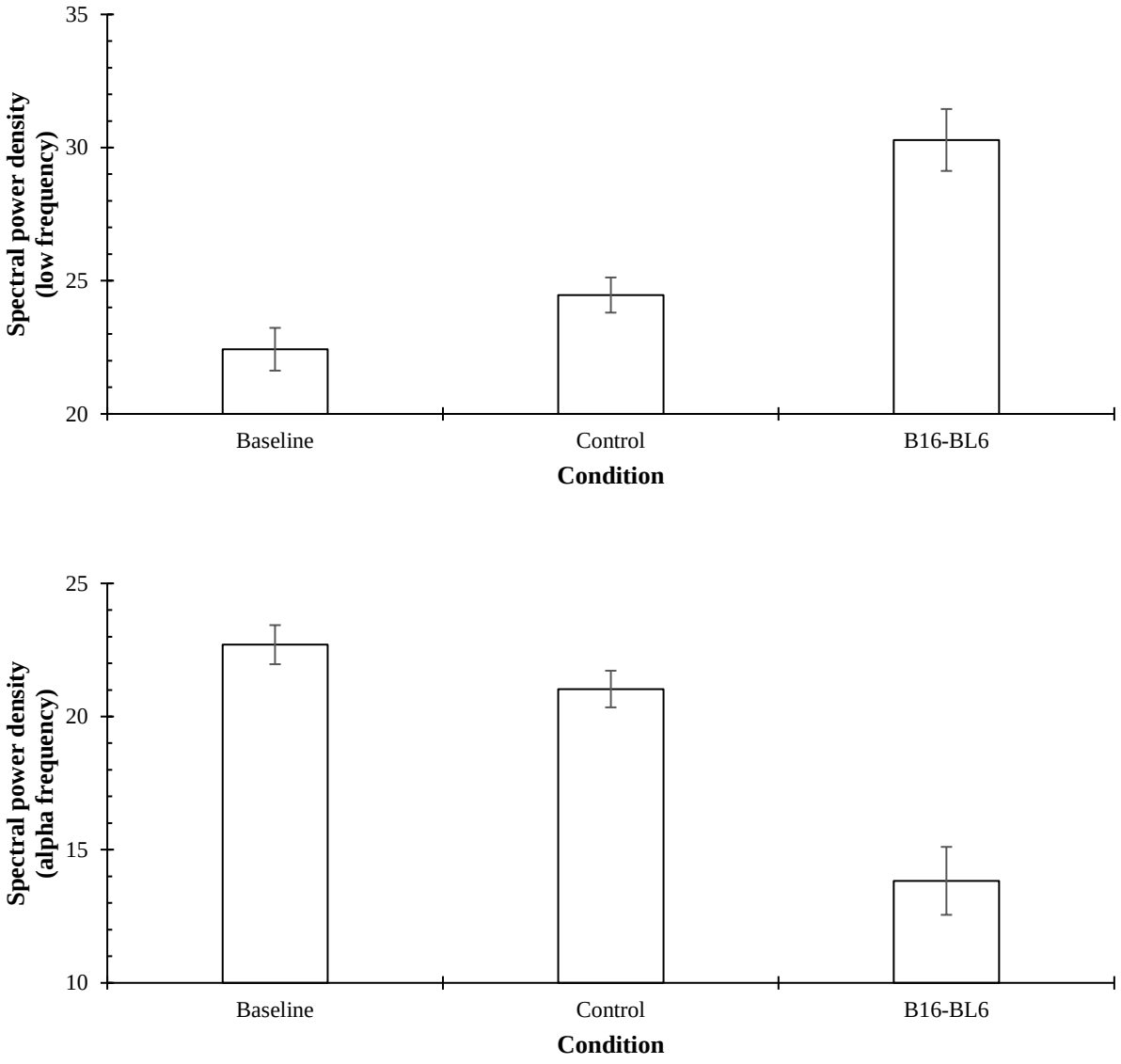

***Figure 3-6*. Comparison of the spectral power density between conditions, in the low frequency bin (upper) and the alpha frequency bin (lower). The sample size for each condition was 5, 34, and 33, respectively. The cell condition was significantly different than the baseline and control conditions; higher in the low frequency bin, and lower in the other six frequency bins. Error bars represent standard error of the mean.**

Comparisons were made between the spectral power density of the B16-BL6 cell line with all non-cancerous cell lines, which included HSG (human submandibular gland), NIH3T3 (murine fibroblast), and C2C12 (murine myocyte) cell lines. Of those, only the C2C12 cell line produced

significant results following spectral power density analysis when compared to the B16-BL6 cell conditions and the control condition. The frequency bin which produced the largest effect size was the mid-beta frequency bin, shown in Figure 3-7. A Kruskal-Wallis ANOVA revealed a significant difference between the two cell conditions and controls ($\chi_{(2)} = 30.094$, $p < .001$, $\eta^2 = .477$); post-hoc tests revealed a significant difference between all conditions.

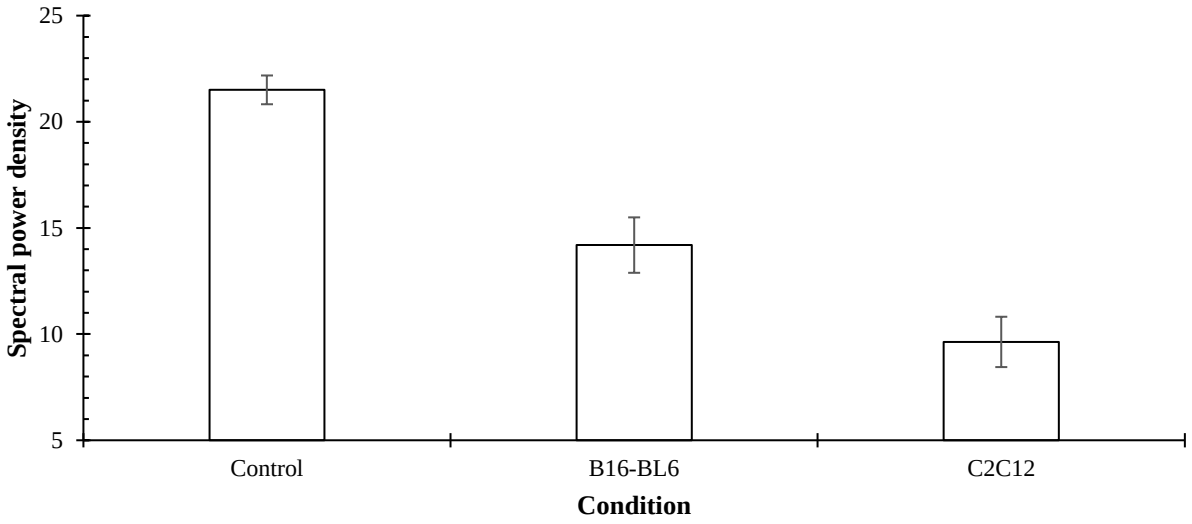

Figure 3-7. **Comparison of the spectral power density of the mid-beta frequency bin between control, B16-BL6, and control conditions. The group sizes for each are 34, 33, and 10, respectively. All three conditions were significantly different from each other. Error bars represent standard error of the mean.**

To further explore this effect, the mid-beta frequency bin was divided into eight 0.5Hz frequency bins (17.00 – 17.49Hz, 17.50 – 17.99Hz… 20.50 – 20.99Hz). Mann-Whitney *U* tests were performed between the B16-BL6 and C1C12 conditions' spectral power density. Only the 19.50 – 19.99Hz frequency bin showed that the spectral power density of the B16-BL6 condition was significantly higher than the C2C12 condition ($U_{(33,10)} = 96.0$, $p = .047$). This is shown in Figure 3-8 below.

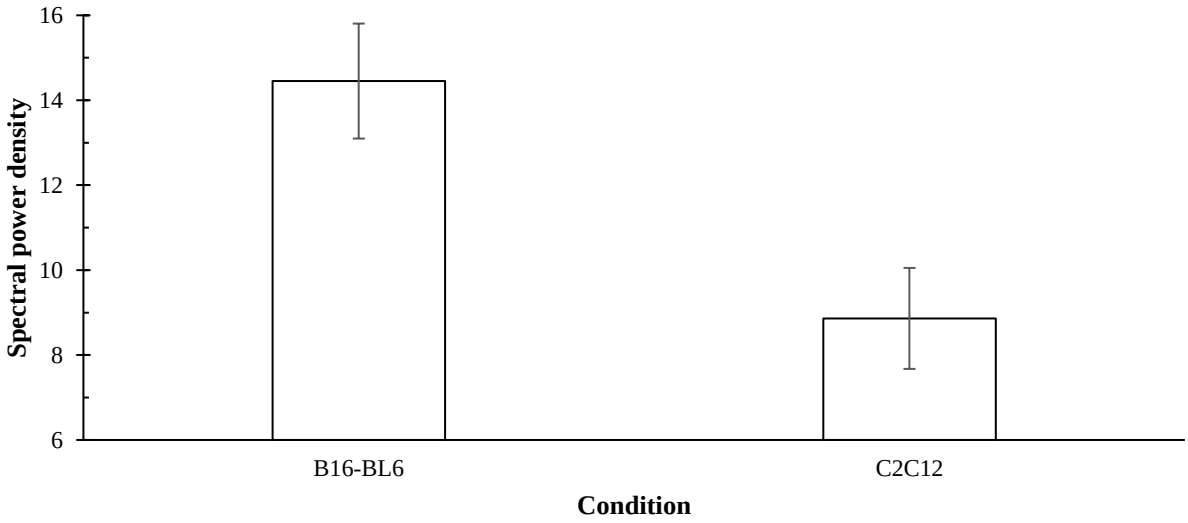

***Figure 3-8*. Comparison of the spectral power density of the 19.50 – 19.99Hz frequency bin between B16-BL6 and C2C12 conditions. Group sizes are 33 and 10, respectively. The B16-BL6 condition was significantly higher than the C2C12 condition. Error bars represent standard error of the mean.**

Discussion

The majority of cell lines were not used in analysis due to low sample sizes. The variability present within each case of the B16-BL6 conditions were considerable, even with a total sample size of 33. As seen in Figure 3-1, there is considerable variation in the conditions with low sample sizes. Minute deviations from the expected procedure due to technical issues, experimental error, etc., may have an impact on the quality of the measurements that can aversely affect the data. This has been noted as a common source of variance between laboratories, specifically when PMTs are involved (Van Wijk, 2005).

B16-BL6 is the main cell line that will be discussed here. This line had the most samples measured (33) out of all cell lines. The B16-BL6 cell line is also the closest line to match the sample size of the control condition (34), which for this experiment was a container with only nutrient agar. Five baseline measurements were also performed, which acted as a measurement of the empty experimental chamber.

A comparison of the mean photon counts between B16-BL6 samples and the non-cell conditions (control and baseline) was performed first, shown in Figure 3-2. As expected, the B16-BL6 samples had significantly higher mean photon counts when compared to controls. This is similar to previous results with B16-BL6 cells and control samples (Dotta *et al*., 2011). Unexpectedly, the baseline condition was not significantly different from either controls or the cell samples. This lack of statistical significance is most likely due to differences in group sizes, approximately 30 measurements each for B16-BL6 samples and controls, but only 5 baseline measurements were performed. An increase in baseline measurements to match the other groups would decrease the variability and most likely be significantly lower than the cell samples, similar to the control. Whether the agar alone is significantly different from the baseline is uncertain.

Next, a comparison of mean photon counts between different container volumes was performed. There were no significant differences between control (agar-only) containers of different volumes. Thus, they were grouped together to compare different container volumes containing the B16-BL6 cell samples.

The three container volumes used (small, medium, and large) showed significantly higher mean photon counts than control samples, but they were not significantly different from each other. The number of cells within each sample was approximately 10^6, regardless of the container (and agar) volume. The volume of agar had no effect on the number of photons that reached the PMT.

In this experiment, the role of the agar was to mimic non-cancerous tissue. Thus, a greater agar volume would be indicative of greater tissue depth. If biophoton emission is to be used as a diagnostic tool, the ability to measure malignant tissue at different depths would be required. The data shown here supports the idea that the mean photon counts detected from a biological sample do not change significantly as a function of agar depth.

The results of Figures 3-4 and 3-5 show that the spectral characteristics of the B16-BL6 samples are also unaffected by agar volume. This was true for all of the frequency bins used: 0-0.9Hz, 1.0-4.9Hz, 5.0-8.9Hz, 9.0-12.9Hz, 13-16.9Hz, 17-20.9Hz, and 21.0-25.0Hz. The frequency ranges of these bins were modelled after typical EEG frequency bins: delta, theta, alpha, and beta. All of the bin sizes were equal, with the exception of the 0-0.9Hz, or "low frequency" bin. The agar depth's lack of impact on the spectral characteristics of the B16-BL6 samples is more support for the ability of biophoton emission to be detected through tissue. These frequencies could be reflective of metabolic processes within the cell (Karbowski 2016).

The spectral densities in the B16-BL6 samples were the inverse of the control samples. For the low frequency bin, the B16-BL6 samples had a greater level of photons than controls; for all other frequency bins, the B16-BL6 samples had a lower level of photon emission than controls. This suggests that there is something unique about the cell samples that is not simply due to the surrounding agar.

Finally, comparisons were made between the B16-BL6 samples and non-cancerous cell lines, or "healthy" cell lines. From all the healthy cell lines tested, only C2C12 cell samples had significantly different spectral densities. These two cell lines are both derived from the same species, *Mus muscularis*, commonly known as the house mouse. This eliminates the issue of species differences, which may have been an issue as cell lines from different species were measured as well.

The statistical difference between the C2C12 condition and the control condition may have been driven by a smaller frequency range. The mean spectral power densities shown in Figures 3-4 through 3-7 were calculated as an average of 240 real data points from the spectral analysis for each case. To accurately inspect within mid-beta frequency bin, mean spectral power densities were calculated from only 30 real data points. This produced eight frequency bins with a width of approximately 0.5Hz. Of these 0.5Hz frequency bins, only the 19.50 – 19.99Hz frequency bin showed a significant difference between B16-BL6 cells and C2C12 cells. A Kruskal-Wallis ANOVA was not used, as it had been demonstrated that there was a significant difference between the B16-BL6 cells and the control condition in higher frequency bins, and that the spectral power density of the C2C12 cell samples tended to be lower than the B16-BL6 cell samples.

It is important to note that there was no statistical difference between the mean photon counts of the B16-BL6 and C2C12 conditions. As the spectral power density data shows, the

measurable differences between the mentioned conditions will not be the number of biophotons released but the frequency patterns that described how they are released. One of the key differences between the two cell lines is their metabolic activity. B16-BL6 cells are cancerous; they will continue to grow and divide until they reach confluence and die. C2C12 cells also divide quite rapidly; however, as the culture approaches confluence the cells will begin to differentiate into myotubules and cease proliferation. Popp has suggested that the number of individuals within a population can lead to constructive and destructive interference patterns (2003). This could lead to variable biophoton emission at different stages of growth.

The B16-BL6 and C2C12 cell lines have different metabolic behaviour. Kim *et al*. have shown that there is a positive relationship between the biophoton emission and the proliferative rate of the HeLa cancer cell line (2007). Metabolism is one of the proposed mechanisms to explain the production of biophoton emission. It is intuitive that metabolic differences would then correlate with changes in biophoton emission frequency. Any factor that could affect metabolism could also affect biophoton emission, such as the number of mitochondria (Rahnama *et al*., 2011). An accurate description of these changes in metabolic activity would require a vast amount of data. These changes in biophoton activity would appear to be very subtle as only two of the observed cell lines were statistically different, with regard to spectral power density.

Differentiation of cancerous and healthy cells with the use of biophoton activity will not be a simple task. Measurements must be consistent and accurate. True discrimination will require large database-levels of information. Spectral analyses, or some other measure of frequency, will be essential as a supplement to mean photon counts. Murugan *et al*. were able to discriminate between cancerous and non-cancerous cell lines with the use of specific wavelength filters (2018). Further research will also have to examine combinations of cell lines or tissue types.

Discrimination in an *in-vivo* setting will also have to account for the combination of tissue types, if biophoton activity is to be used as a diagnostic tool.

References

Dotta, B. T., Buckner, C. A., Cameron, D., Lafrenie, R. M., Persinger, M. A. (2011). Biophoton emissions from cell cultures: biochemical evidence for the plasma membrane as the primary source. General Physiology and Biophysics; 30: 301-309.

Karbowski, L. M., Murugan, N. J., Persinger, M. A. (2016). Experimental evidence that specific photon energies are stored in malignant cells for an hour: the synergism of weak magnetic field-LED wavelength pulses. Biology and Medicine; 8(1): BM-162-16.

Kim, J., Kim, Y., Lee, Y. J., Kobayashi, M., Tsutsumi, Y., Kondo, R., Lee, S. K., Soh, K. (2007). Spontaneous ultraweak photon emission during the growth of the cell population HeLa cell line. Journal of Health Science; 53(4): 481-485.

Murugan, N. J., Rouleau, N., Karbowski, L. M., Persinger, M. A. (2018). Biophotonic markers of malignancy: discriminating cancers using wavelength-specific photons. Biochemistry and Biophysics Reports; 13: 7-11.

Popp, F. A. (2003). Properties of biophotons and their theoretical implications. Indian Journal of Experimental Biology; 41: 391-402.

Popp, F. A. (2009). Cancer growth and its inhibition in terms of coherence. Electromagnetic Biology and Medicine; 28: 53-60.

Rahnama, M., Tuszynski, J. A., Bokkon, I., Cifra, M., Sardar, P., Salari, V. (2011). Emission of mitochondrial biophotons and their effect on electrical activity of membrane via microtubules. Journal of Integrative Neuroscience; 10(1): 65-88.

Takeda, M., Tanno, Y., Kobayashi, M., Usa, M., Ohuchi, N., Satomi, S., Inaba, H. (1998). A novel method of assessing carcinoma cell proliferation by biophoton emission. Cancer Letters; 127: 155-160.

Takeda, M., Kobayashi, M., Takayama, M., Suzuki, S., Ishida, T., Ohnuki, K., Moriya, T., Ohuchi, N. (2004). Biophoton detection as a novel technique for cancer imaging. Cancer Science; 95(8): 656-661.

CHAPTER FOUR

EXPOSURE OF B16-BL6 CELL CULTURES TO EXTREMELY LOW-FREQUENCY ELECTROMAGNETIC FIELD ALTERS BIOPHOTON EMISSION AS A FUNCTION OF DISTANCE

Abstract

Biophoton emission is produced by all living systems; this emission pattern has been shown to be altered by the presence of an electromagnetic field (EMF). Cultures of B16-BL6 cells were exposed to a weak EMF produced by a specially constructed EM generator, called the 'Resonator', for one hour. This EM generator incorporates multiple geometric ratios in its design, including the golden ratio (phi), pi, root 2, root 3, and root 5, and has been used to purify water of toxins. There was a significant decrease in mean photon counts from B16-BL6 cells exposed at a distance of 1m compared to those exposed at 0m. Alterations in the spectral power density variability were also observed in the 8-10Hz range. The EM generator may have an impact on the viability of the exposed cell cultures, but only at specific distances.

Introduction

All living biological systems emit low levels of electromagnetic radiation, referred to as biophoton emission. It has been proposed that metabolic activity is the primary source of biophoton emission, specifically the oxidation of free radicals. This is supported by experimental evidence including the addition of hydrogen peroxides to tissue, or a reduction of tissue antioxidants (Alipour, 2015). This proposed mechanism is the same for both types of biophoton emission: spontaneous and induced. Spontaneous biophoton emission is produced by the natural metabolic activity of the organism. Induced biophoton emission is observed after an organism has been exposed to one or more of several factors, which can be natural or artificial. These factors include bacterial or viral infection, stress, external temperature, and ionizing radiation, to name a few (Cifra & Pospisil, 2014). Electromagnetic fields (EMFs) have also been previously demonstrated to induce biophoton emission in combination with specific wavelengths of light (Karbowski *et al.* 2016).

The examination of the effect of exposure to an EMF alone on biophoton emission has not been done. In the experiment by Karbowski and colleagues, three complex, time-varying EMF patterns were used (2016). The central effect observed was the increase in mean photon counts when B16-BL6 cells were exposed to a combination of both an EMF and 450nm light. In this experiment, the aim was to focus on the characteristics of a single EMF.

The EMF used in this experiment, was generated by a novel device invented by Dr. K. Shallcross. This EM generator, referred to as the 'Resonator', uses a series of metallic roller magnets arranged at specific angles to create a pattern derived from a sacred geometry. Sacred geometry refers to numerical values derived from simple geometric relations, such as π, √2, √5,

and φ (Shallcross, 2016). When the device is on, the roller assemblies rotate, which generates a weak EMF, in the range of 1μT to 10μT. When the device is off, no EMF is generated.

Previous experiments with the 'Resonator' have shown differential effects on the growth rate of bacterial cultures (Tessaro *et al*., 2015a). Three of the examined bacteria species were shown to have an increased growth rate after exposure, while one species had a decreased growth rate. This suggests that the complexity of the 'Resonator' field is such that it can have opposite effects on different species. This is most likely mediated via different effects on cellular metabolic pathways.

In addition, exposure of the 'Resonator' EMF to B16-BL6 cells for three hours has been shown to significantly decrease cell viability (Tessaro *et al*., 2015b). Takeda and colleagues have demonstrated that biophoton emission in cancerous cells was related to the population size (Takeda *et al*., 1998). Thus, if exposure to the 'Resonator' EMF is correlated with decreased cell viability, and biophoton emission is correlated with cell population, there should be an observable decrease in the biophoton emission of exposed cell cultures.

Methods

B16-BL6 cell cultures were grown in DMEM supplemented with 10% fetal bovine serum and 1% antibiotics on 10mm Petri dishes and allowed to reach confluence in a water-jacket copper-lined incubator at 37°C (95% air, 5% CO_2). Cell cultures were grown on Petri dishes in groups of 16. Once confluent, the plates were removed from the incubator and placed into one of four conditions: Field 1m, Field 0m, Sham 1m, Sham 0m. Each condition contained four cell plates. These conditions refer to the state of the 'Resonator', either with an active magnetic field in the Field conditions, or with the device turned on but with no movement of the coils; these were the Sham conditions. The cell plates were placed on a table at either 1m or 0m from the 'Resonator'. Vertically, the cell plates were separated from the 'Resonator' by a distance of approximately 10-15cm. The distance of vertical separation was constant between the 1m and 0m conditions. The plates were arranged side by side, such that they formed an approximate square on the surface of the table. This table did not make physical contact with the 'Resonator' to remove any vibrational effects from the spinning of the 'Resonator' coils. The 'Resonator' was then turned on for the Field conditions. Cell plates were left on the table for no less than one hour.

Once the exposure was complete, the biophoton output of the cell plates was immediately measured. This measurement was performed in the experimental chamber, a specially constructed space measuring 1.32m long, 1.27m wide, and 1.70m in height. Before the experimental chamber was opened, a baseline measurement was taken. The cell plate was placed on a small polystyrene box, which is located on a chair in the centre of the experimental chamber. The sample was measured with four PMTs, which were located on three walls and the ceiling of the experimental chamber. Each PMT was separated from the sample by a distance of 15cm. A one-minute delay between the closure of the experimental chamber and the start of the measurement ensured minimal

light contamination of the cell plate measurement. Each cell plate was measured for two minutes. Including the one-minute delay, each plate was in the experimental chamber for three minutes. After each plate had been measured, a post baseline measurement was performed. This procedure was identical to the cell plate measurement, including the one-minute delay. Field and sham measurements for plates exposed at any given distance were recorded on the same day to minimize daily variations.

Data from the measurement sessions was exported into IBM SPSS for statistical and spectral analyses.

Results

All of the analyses below were performed with the data from the PMT located at the Head position. Only the second minute of the measurement was used for analyses. A total of 14 cases from two different days were removed due to outlier effects. This gave a total *N* of 41.

First, a comparison was made between the baseline condition and all cell conditions. A Student's *t* test for independent samples revealed a significant difference between the baseline and the cell conditions (t = -6.260, $p < .001$, $\eta^2 = .445$). The cell condition had significantly higher mean photon counts than the baseline condition. This is shown in Figure 4-1.

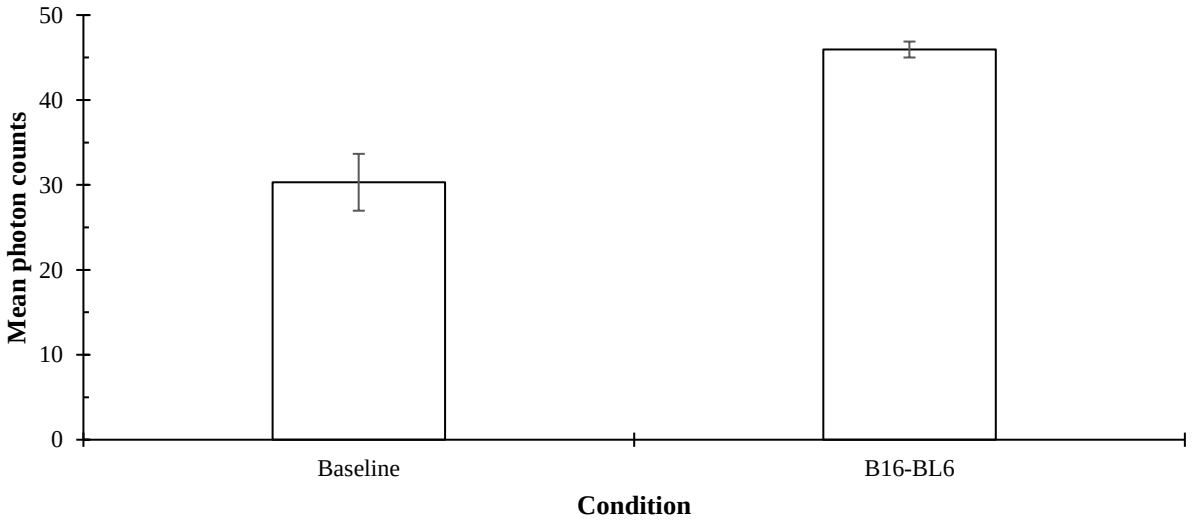

***Figure 4-1*. Comparison of the mean photon counts between the baseline condition and all B16-BL6 conditions. The B16-BL6 condition was significantly higher than the baseline condition. Error bars represent standard error of the mean; *n* = 5 for baseline, *n* = 36 for B16-BL6.**

To expand on the B16-BL6 conditions, a pair of Student's *t* tests for independent samples were used to compare mean photon counts between field condition and distance. This is shown in Figure 4-2 below. There was no significant difference in mean photon counts between the Sham 0m condition and the Sham 1m condition ($t_{(14)} = 0.163$, $p = .873$). The mean photon counts of the

Field 1m condition were significantly lower than the Field 0m condition ($t_{(18)} = 2.120$, $p = .048$, $\eta^2 = .200$).

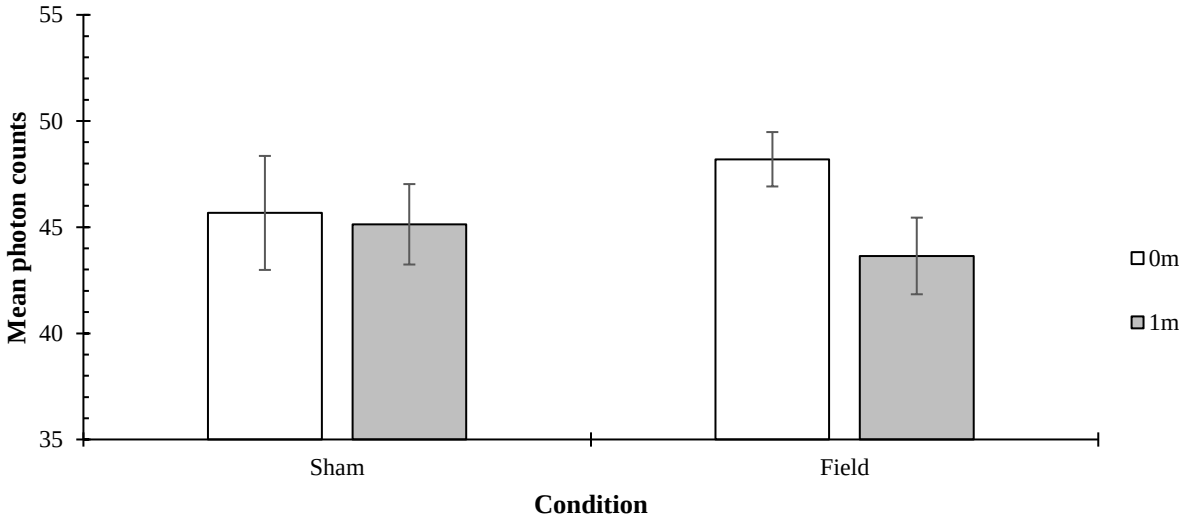

***Figure 4-2*. Comparison of the mean photon counts between Sham and Field conditions at 0m and 1m. There was no significant difference between the Sham 0m and Sham 1m conditions; the Field 1m condition was significantly lower than the Field 0m condition. Error bars represent standard error of the mean; the *n* for each condition was 8, 8, 12, and 8, respectively.**

A series of one-way ANOVAs were performed to compare the spectral power density variability by condition membership. The spectral power density variability is the standard deviation of the spectral power density, per case. For this procedure, the spectral power density variability was averaged into multiple frequency bins, each with a width of 1-4Hz. The only bin that produced a significant main effect was the low alpha (8-10Hz) frequency bin ($F_{(3,32)} = 4.047$, $p = .015$, $\eta^2 = .255$). Post-hoc tests revealed the effect was being driven by the difference between the Sham 1m and Field 1m conditions ($p < .05$). This is shown in Figure 4-3 below.

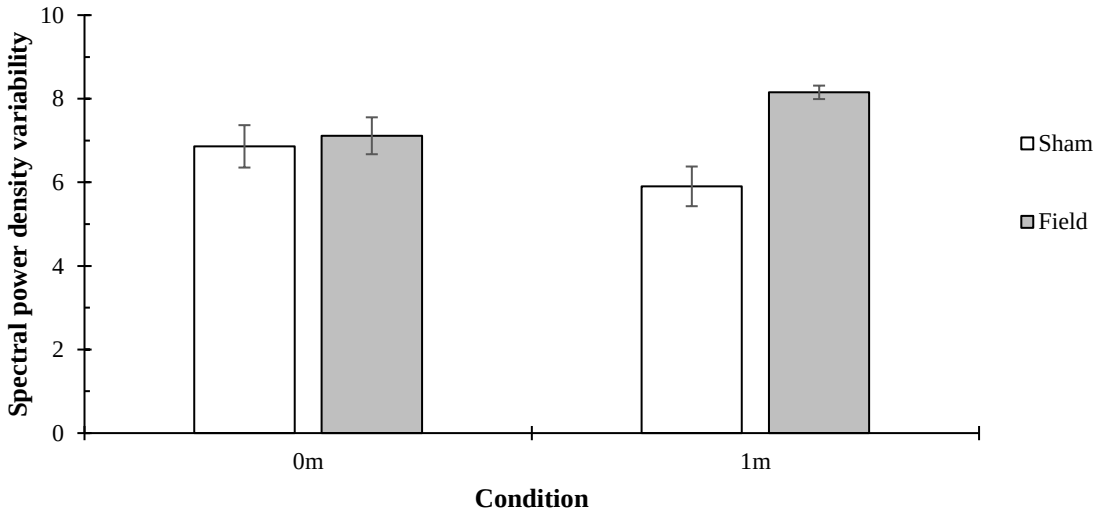

***Figure 4-3*. Comparison of the spectral power density variability in the low alpha frequency bin. There was a significant difference between the four conditions; post-hoc tests revealed the significance was being driven by the Sham 1m and Field 1m conditions. The Field 1m condition spectral power density variability was significantly higher than the Sham 1m condition. Both Sham and Field 0m conditions were not significantly different from any other conditions. Error bars represent standard error of the mean; the *n* for each condition was 8, 8, 12, and 8, respectively.**

Discussion

The mean photon counts from B16-BL6 cell cultures were significantly higher than the baseline, regardless of experimental conditions. It is evident that the presence of a biological system increases the mean photon count as measured by the PMT. This is consistent with previous findings.

A comparison of mean photon counts between the experimental conditions revealed novel results. The sham conditions were not significantly different from one another, which would be expected. The proximity to the static EMF produced by the 'Resonator', while inactive, did not seem to impact the mean photon counts.

The field conditions exhibit distance effects. The Field 1m condition had significantly lower mean photon counts compared to the Field 0m condition. There are three possible interpretations: one, the induced EMF from the 'Resonator' at 0m led to increased mean photon counts in the Field 0m condition; two, the induced EMF from the 'Resonator' at 1m led to decreased mean photon counts in the Field 1m condition; three, a combination of effects one and two. Another factor to be considered is the impact of vibrational effects. Vibrational effects have been implicated in a possible mechanism of 'Resonator'-cell interaction (Tessaro, 2015b). Thus, vibrational effects may interpose themselves with the EMF effects while the 'Resonator' is active; however, the plates were not placed in direct contact with the 'Resonator' to minimize the effects of vibration.

The spectral power density of the four conditions were compared with a series of one-way ANOVAs; no significant differences were found. The variability of the spectral power density was also compared in a similar fashion; a significant effect was found in the low alpha frequency bin.

This frequency bin was an average of the spectral power density variability scores in the 8-10Hz frequency range. Post-hoc Tukey HSD revealed that the effect was driven by the Sham 1m and Field 1m conditions. The spectral power density variability was significantly higher in the Field 1m condition compared to the Sham 1m condition.

The implications of these two effects, mean photon counts and spectral power density variability, are centred on the Field 1m condition. This condition had significantly lower mean photon counts than the Field 0m condition, and significantly higher spectral power density variability than the Sham 1m condition. This implies fewer photons are being released and the rhythmicity of photon emission in the 8-10Hz range is less consistent. If the B16-BL6 cells' metabolic activity is altered by the generated EMF, this may explain why the counts were lowered in the Field 1m condition. The EMF that is generated is an extremely low-frequency field which does not change as a function of position (relative to the 'Resonator'), although the intensity decreases with distance. This is a significant quality, since the signal pattern and frequency remain consistent over distance, the only EMF factor that would change between the Field 0m and Field 1m conditions is the intensity of the field.

It is important to mention that the spectral power density variability of the Sham conditions were not significantly different from the spectral power density variability of the Field conditions. This may be a function of low sample size. High variability is also a common issue when biophoton emission is involved. This is often why spectral analyses are performed. The cell cultures were removed from the incubator for exposure and for measurement. Ideally, both of these processes should be carried out in a temperature-controlled environment.

This experiment was meant to act as a brief observation of the biophoton emission patterns produced by the B16-BL6 cell cultures. As such, no cellular observations were made. Future

experiments should include cell counts and viability. Comparisons could then be made directly between cellular variables and measured photon activity.

References

Alipour, A. (2015). Demystifying the biophoton-induced cellular growth: a simple model. Journal of Advanced Medical Sciences and Applied Technologies; 1(2): 112-115.

Cifra, M., Pospisil, P. (2014). Ultra-weak photon emission from biological samples: definition, mechanisms, properties, detection and applications. Journal of Photochemistry and Photobiology B: Biology.

Karbowski, L. M., Murugan, N. J., Persinger, M. A. (2016). Experimental evidence that specific photon energies are stored in malignant cells for an hour: the synergism of weak magnetic field-LED wavelength pulses. Biology and Medicine; 8(1): BM-162-16.

Shallcross, K. (2016). *Canadian Patent No. CA 2631215*. Ottawa, ON: Canadian Intellectual Property Office.

Takeda, M., Tanno, Y., Kobayashi, M., Usa, M., Ohuchi, N., Satomi, S., Inaba, H. (1998). A novel method of assessing carcinoma cell proliferation by biophoton emission. Cancer Letters; 127: 155-160.

Tessaro, L. W. E., Murugan, N., Persinger, M. A. (2015)a. Bacterial growth rates are influenced by cellular characteristics of individual species when immersed in electromagnetic fields. Microbiological Research; 172: 26-33.

Tessaro, L. W. E., Karbowski, L. M., Lafrenie, R. M., Persinger, M. A. (2015)b. Application of dynamic magnetic fields to B16-BL6 melanoma cells linked with decrease in cellular viability after short exposures. Journal of the International Association of Advanced Technology and Science; 1: 1-11.

CHAPTER FIVE

DISCUSSION

The results of the previous experiments have all demonstrated that unique biophoton emission is associated with biological systems. The presence of a biological system, including a human or a cell culture, is enough to produce significant changes in measured photon activity. Mean photon counts were the most apparent difference between biological and baseline samples. This is reflective of the overall intensity of the measured subject; more measured photons, more released photons, more light, higher intensity.

The appearance of intensity effects is not novel for living systems. Even as early as 1975, inanimate objects were shown not to increase photon counts (Van Wijk, 2005). There is something unique about biological systems that produces an increase in mean photon counts. The most probable source of this increased photon activity is metabolism. Specifically, biophoton emission is thought to be associated with the oxidation of electronically exited species (Cifra, 2014). Increases in metabolic activity should lead to increases in biophoton emission.

While that may be true in theory, in practice it is difficult to observe. As seen in Chapter 3, no intensity differences were observed between B16-BL6 and C2C12 cell lines. While both cell lines are metabolically active, intensity alone was not able to differentiate between the two. The two human individuals in Chapter 2 also did not present statistically different intensities of photon emission. This does not indicate that metabolic activity has no impact on intensity, but rather that the metabolism of the organisms compared must be substantially different in order for differences to be measurable. Metabolism does not appear act as a method of differentiation on its own.

Information must be gleaned from other areas. The previous experiments show that it is the *pattern* of photon activity, rather than mean photon counts, that are able to account for the differences between organisms. Significant spectral power density differences were present in all three experiments. Spectral power density is reflective of the underlying patterns present within a

time-series. These patterns may or may not be apparent on visual inspection of the data, or may require Fourier transformation to be detected.

Difficulty comes in the interpretation of the spectral power density. The observable frequencies are restricted by the Nyquist limit, effectively half the sampling rate of the measurement device. The PMTs used in this experiment were set to their maximum sampling rate, 50Hz; thus, the highest observable frequency from spectral analysis was only 25Hz. Ideally, the use of a PMT with a higher sampling rate would be able to provide higher frequencies that was not detectable in this experimental set-up.

Another important distinction to make is that the frequencies in question are not indicative of the frequency of the photons that interact with the PMT; rather, they represent the rhythm by which photons interact with the PMT. A significant frequency band in the 8-10Hz range indicates that photons interact with the PMT 8-10 times per second, not that the photon itself has an 8-10Hz frequency. Thus, these frequencies are more indicative of biological rhythms like those associated with metabolism. These patterns may be related to intercellular communication. Research by Dotta and colleagues has shown that the introduction of epidermal growth factor in B16-BL6 cell cultures increased the biophoton emission energy intensity (2011). Photons can degrade cryptochrome, a protein involved with maintaining the calcium-calmodulin complex that regulates cell division (Alipour 2015).

Different frequency bins of photon emission were revealed as significant in each experiment. In Chapter 2, these bins were in the alpha (7-10Hz) and delta (0-3Hz) range; in Chapter 3, it was the 19.50 -19.99Hz bin; in Chapter 4 the significant bins were again in the alpha range. It was unlikely that one frequency bin would be found that would be able to explain all of the

differences between species or individuals. There exists an incredible amount of diversity within and between individuals; diversity would be expected in these observed frequency bins as well.

One important result that was consistent across experiments was that the changes in the frequency of the released photons between organisms/individuals are significantly different without an increase in mean photon counts. With metabolism as the primary source of biophoton emission, this would indicate that the total amount of energy within an individual is conserved; the difference is related to how that energy is allocated to various functions that alters the frequency of the emitted photons. This would not be the case if the mean photon counts also increased between organisms, as that may account for frequency increases.

One issue that arose in the data analysis of the experiments was the presence of delayed luminescence. This effect is observed immediately following exposure to sources of artificial light, even at low intensities. To avoid this effect, the measurement of all experiments was delayed by one minute after the subject was placed within the experimental chamber. For Chapter 4, analysis was only conducted on the second minute of the recording, or three minutes after the subject was placed within the chamber. The second technique seems to be the most consistent technique in terms of the reliability of the mean photon counts. Future studies should aim to either: delay the measurement until reasonably consistent photon counts are achieved; or, segment the recording such that only the segments with consistent photon counts are analyzed. Methods such as those used by Popp and Yan to mathematically explain delayed luminescence could also be employed (2002).

It was observed that the spectral results of photon emission divided into frequency bins had the most relevance to the discrimination of healthy and unhealthy tissues. The detection of biophoton emission is non-invasive and could be developed as a diagnostic tool. The largest

logistical challenges would include the set-up of PMTs and the required dark environment. If there is a biophoton activity that can be identified and associated with various cancers, then regular diagnostics and early detection of cancer could be achieved. The use of induced biophoton emission could also be used as a diagnostic if populations of cells respond to the same stimulus in the same fashion. Murugan and colleagues have demonstrated specific biophoton peaks released in response to the specific coordination of light and an EMF (2018).

These results showed that discrimination between organisms is possible with the use of only biophoton emission data. With enough research, biophoton emission may become a realistic alternative for the early detection of various diseases.

References

Alipour, A. (2015). Demystifying the biophoton-induced cellular growth: a simple model. Journal of Advanced Medical Sciences and Applied Technologies; 1(2): 112-115.

Cifra, M., Pospisil, P. (2014). Ultra-weak photon emission from biological samples: definition, mechanisms, properties, detection and applications. Journal of Photochemistry and Photobiology B: Biology.

Dotta, B. T., Buckner, C. A., Cameron, D., Lafrenie, R. M., Persinger, M. A. (2011). Biophoton emissions from cell cultures: biochemical evidence for the plasma membrane as the primary source. General Physiology and Biophysics; 30: 301-309.

Murugan, N. J., Rouleau, N., Karbowski, L. M., Persinger, M. A. (2018). Biophotonic markers of malignancy: discriminating cancers using wavelength-specific biophotons. Biochemistry and Biophysics Reports; 13: 7-11.

Popp, F. A., Yan, Y. (2002). Delayed luminescence of biological systems in terms of coherent states. Physics Letters A; 293: 93-97.

Van Wijk, R., Van Wijk, E. P. A. (2005). An introduction to human biophoton emission. Forsch Komplementarmed Klass Naturheilkd; 12: 77-83.

APPENDIX

LIST OF ABBREVIATIONS

4T1:	4T1, murine breast cancer
AsPC:	AsPC-1, human pancreatic cancer
B16-BL6:	B16-BL6-C57bl, murine melanoma
C2C12:	murine myocyte
DMEM:	Dulbecco's Modified Essential Medium
EMF:	electromagnetic field
HSG:	human salivary gland
MCF-7:	human breast cancer
NIH-3T3:	mouse mesenchymal tissue
PMT:	photomultiplier tube
UPE:	ultra-weak photon emission